United States
Department of
Agriculture

**Agricultural
Research
Service**

March 1994

U.S. Dairy Forage Research Center 1993 Research Summaries

U.S. Dairy Forage Research Center. 1994. Research Summaries. U.S. Department of Agriculture, Agricultural Research Service.

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> 1925 Linden Drive West
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Preface

It is a pleasure to update our progress by bringing you these summaries of recent research. The U.S. Dairy Forage Research Center is a unique part of the national research program of the Agricultural Research Service, U.S. Department of Agriculture. The Center's mission is to build a knowledge and technology base for the dairy industry to fully exploit the use of forages in the production of milk. The Center has agricultural engineers, plant and soil scientists, microbiologists, ruminant nutritionists and a chemist working together to increase the efficiency of forage production and utilization by dairy farmers. We function in close cooperation with the Agricultural Experiment Stations of several states. The Center is located on the campus of the University of Wisconsin, Madison, and has "Cluster" locations in St. Paul, MN, Ames, IA, East Lansing, MI, and Ithaca, NY. The Center's research farm with facilities for 300 milking cows is located on 63 acres of USDA land on the banks of the Wisconsin River in Prairie du Sac, WI. An additional 1200 acres of adjacent land is utilized by the Center by agreement with the U.S. Department of the Army. The Center was established in 1980 and has made steady growth since. At present there are fifteen scientists: ten at Madison, and one at each of three Cluster locations, and two at the St. Paul, Minnesota Cluster location. Scientists hold faculty appointments in university departments and provide supervision for approximately 15-20 graduate students and 5-10 post doctoral fellows.

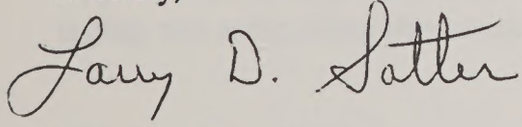
We have added Dr. Rao Kanneganti to the systems work group this past year. Rao did graduate and post-doctorate study at Cornell University. Dr. Kanneganti will be developing crop growth models that will be incorporated into DAFOSYM, the Dairy Forage Systems Model used for computer simulation of a dairy farm. Dr. Al Rotz has recently added a segment to DAFOSYM to evaluate dairy manure systems.

We initiated a grazing research program this past year. It had a modest beginning last summer with about fifty heifers distributed in five treatment groups. Dr. Kanneganti, working with the staff, conducted the study. This coming summer we will start a longer term grazing study with about 50 lactating cows that will be on a seasonal calving program. There is great interest in grazing as a management option for lowering cost of milk production.

Last year we highlighted progress of the cell wall work group. This year we are highlighting the progress made in the engineering area, with special emphasis on the forage mat technology developed at the U.S. Dairy Forage Research Center. This 'radical approach' to forage harvest is stirring much interest, particularly in Northern Europe. We are excited about prospects for this new approach to forage harvest.

We are pleased and very proud of the way Center scientists from diverse disciplines interact and bring their collective insights to bear on the problems of forage production and utilization. This collection of research summaries illustrates the progress they are making in developing information to help dairy farmers utilize their resources more effectively. The research is intended to benefit producers of forage crops, dairy farmers and the consumers of dairy products.

Sincerely,



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Acknowledgment

Appreciation is expressed to Gloria Palmer for her interest in and dedication to the task of typing and assembling this annual research summary.

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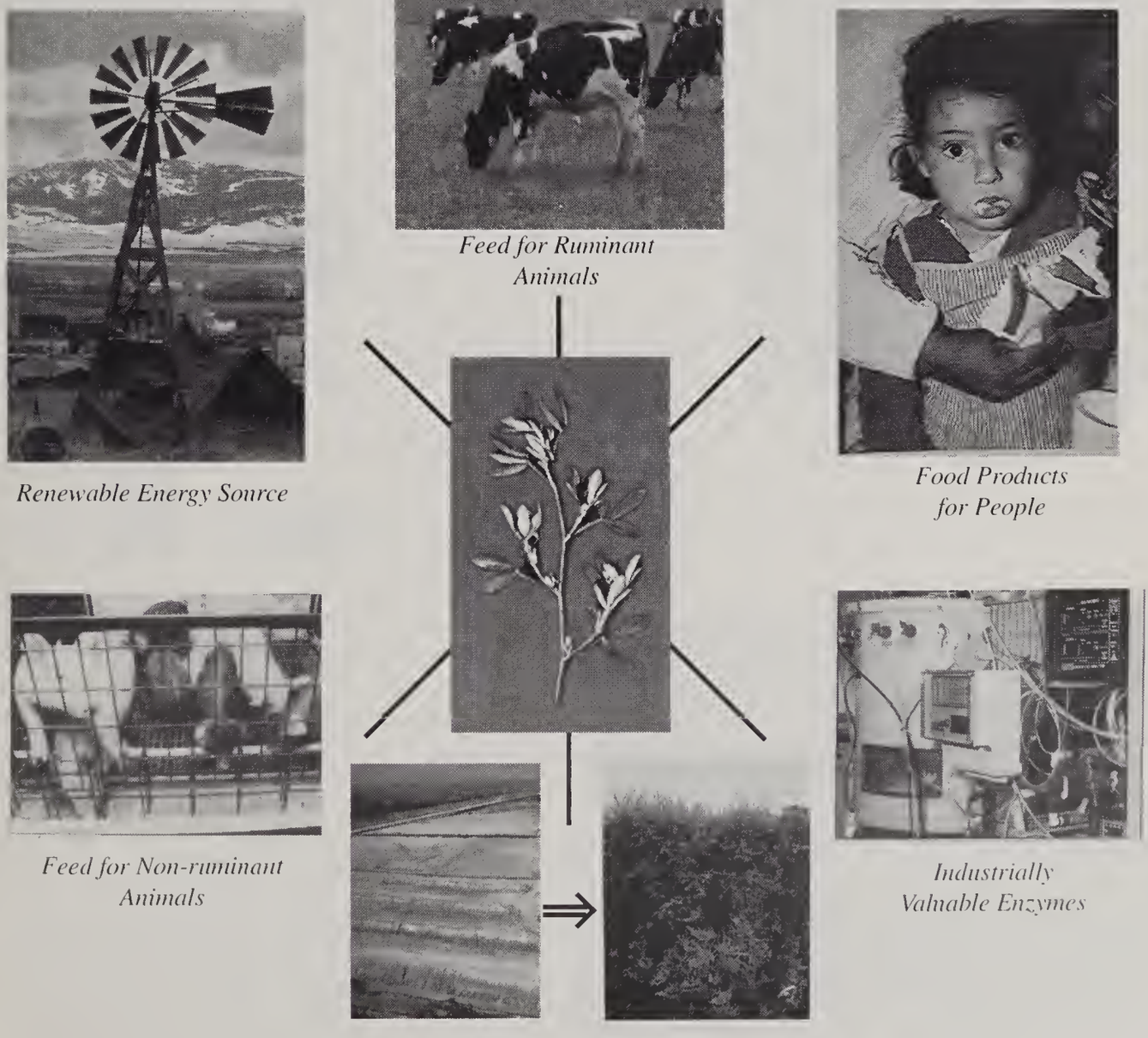
PUBLICATIONS

Increasing the Value and Versatility of Forage Crops Through Processing

The high per acre protein and energy yields of forage crops, as well as their conservation of soil, water, and non-renewable energy, are generally appreciated. Their value and acreage, however, have been limited by two major factors: 1) value reduction by significant quality and quantity losses during harvesting and storage, and 2) the intimate association of more readily digestible constituents with large quantities of fiber, largely limiting their use to ruminant rations.

There now appears to be potential for retaining and even improving the feeding value of forage crops during harvest and storage. In addition, it is possible to create "value-added" products and to increase the total value and versatility of the crop when herbage is separated into fractions having significant applications. Which of the numerous potential products (Fig. 1) will find practical, large-scale use will depend on many factors. However, innovative research leading to valuable end-products while minimizing processing costs will be indispensable.



Feed for Ruminant Animals

Renewable Energy Source

Food Products for People

Feed for Non-ruminant Animals

Industrially Valuable Enzymes

Bioremediation of Toxic Sites

Fig. 1: Processing forage crops can lead to a wide variety of applications.

Forage Mat Technology: Reducing Harvesting Losses While Increasing Feeding Value

Losses of quality and quantity occur in a number of ways during forage harvest including rain damage, plant respiration, and leaf shatter. The forage mat process was developed in an attempt to reduce these losses by increasing the rate and uniformity of field drying. The process consists of four steps carried out by a single machine: (1) mowing, (2) severe conditioning which reduces stems to fibrous splinters and mashes softer plant parts, (3) pressing the conditioned forage into thin, cohesive mats, and (4) returning the mat to the stubble for drying (Fig. 2). The conditioning process greatly increases the specific surface area of the forage, causes it to absorb more solar energy, and reduces resistance to moisture egress as it is vaporized by the absorbed energy. The mat structure aids retention of particles created by conditioning and appears to enhance conductivity of heat and moisture within the mat leading to faster and more uniform drying.

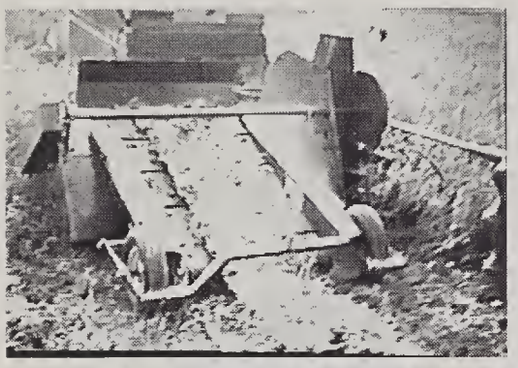
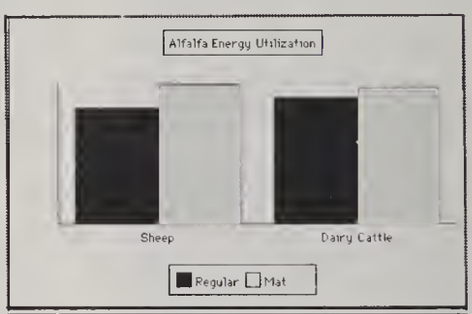


Fig. 3 TOP: Mats being chopped for silage
BOTTOM: Mats being baled for hay

Fig.2 Forage mat dried in field



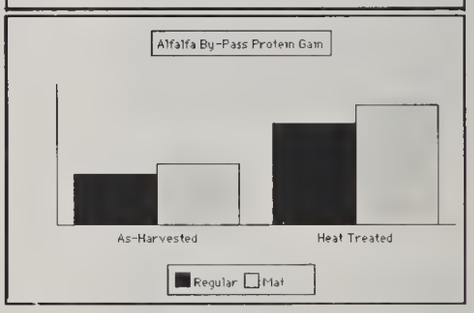


Fig. 4 TOP: Sheep/Cattle trials
Fig. 5 BOTTOM: Estimated bypass protein

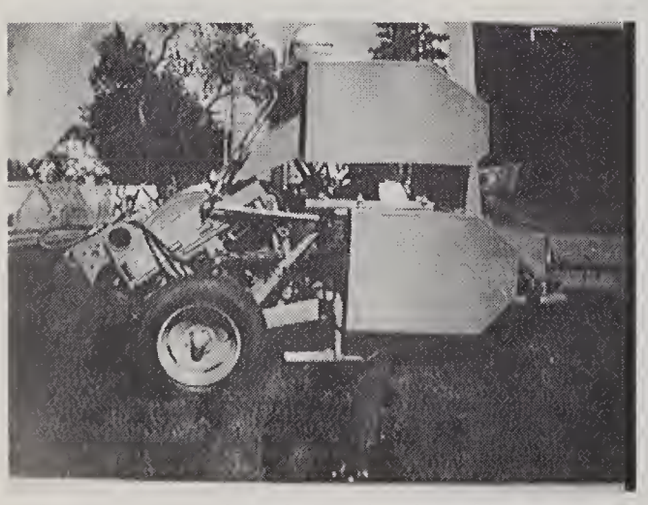


Fig 6: Mat machine being developed by USDFRC at the University of Wisconsin, Madison

Under good drying conditions, mats may dry to ensiling moisture in 2-3 hours and to baling moisture in 6-8 hours allowing them to be removed from the field before nightfall (Fig. 3). The same properties which cause forage mats to dry rapidly lead to leaching losses 3-5 times those of unconditioned forage if rained on. Probability of rain occurring is drastically reduced by the short exposure time, however.

Fig. 7: Press for expressing alfalfa juice

Feeding trials with sheep, goats, and cattle have consistently shown that more energy was derived from mat-processed forage than from conventional forage. Increases typically ranged from 11 to 18% (Fig. 4). Scanning electron microscopy shows many more sites colonized by rumen micro-organisms apparently due to the structural damage caused by severe conditioning. In vitro estimates of "by-pass" protein levels in mat-processed hay and conventional hay have shown the former to be about 21% higher (Fig. 5). It is believed that the Maillard reaction between sugars and proteins in the alfalfa juice is responsible for the more resistant protein in the mat hay.

The work on forage mat technology at Madison has inspired similar work at more than ten locations in Europe, Canada, and the U.S. by both industrial and research groups. These efforts could lead to a number of variants of the process as contenders in the market place (Fig. 6).

Wet Fractionation: Tailoring Forage-Derived Products to New Applications

By expressing juice from fresh herbage, 25 - 35% of the most valuable dry matter can be separated from the fiber which remains a high quality ruminant feed or a source of ligno-cellulosics for further processing (Fig. 7).

The juice, which is essentially fiber-free, can be grossly divided into three major fractions: (1) a particulate or chloroplastic protein concentrate, rich in chlorophyll and pigments, (2) a soluble protein fraction, and (3) a soluble fraction containing sugars and salts. The two protein fractions can be heat precipitated together (Fig. 8). This is currently done on an industrial scale in France for livestock feed. The resulting concentrate is the rough equivalent of soybean meal and is priced accordingly. However, when used in the poultry industry for its pigmenting properties, it has commanded triple the price of soybean meal. It has also been produced in "kitchen-scale" installations for supplementing the diets of protein-deficient children world-wide. Researchers at the U.S. Dairy Forage Research Center and University of Wisconsin developed processing equipment now being used in more than 20 Mexican villages. Although the weight of the soluble protein fraction is about half that of the particulate fraction in a typical separation, its value may prove to be 20 times as great. This is because it can be potentially incorporated into a variety of food products such as beverages, soups, sauces, and bakery products. Despite the fact that the dry weight of this fraction may be only 2-3% of the original crop, its value as a food product may be 2-3 times that of the original crop used for traditional purposes.

Fig. 8: Protein concentrate from heat treated juice

Fig. 9: Alpha Amylase Test: Missing white circles show alpha amylase gene has been successfully inserted into alfalfa plant

Biotechnologists at the University of Wisconsin and elsewhere have demonstrated that insertion of genes into forage crops, such as alfalfa, can cause them to produce proteins not ordinarily produced. This has led to the concept of forage crops being used as "factories" to produce valuable industrial substances such as enzymes which, to date, have typically been produced in fermentation vats by microorganisms.

The joint effort of scientists at the University of Wisconsin and U.S. Dairy Forage Research Center is based on the belief that, in the future, enzymes will be used in unprecedented quantities for a variety of applications, including biopulping of wood in the paper industry, hydrolysis of biomass to liquid fuel, bioremediation of contaminated sites, food processing, feed additives, and in cleaning agents. Extraction of the target enzymes from the herbage would be from expressed juice (Fig. 9). The steps in enzyme recovery from the juice include: 1) gentle heat treatment followed by centrifugation to remove particulates, 2) ultra-filtration to concentrate the soluble protein, and removal of the target enzyme from the soluble protein by affinity column or similar means. It is anticipated that, in addition to juice expression, at least the first two separation steps would be carried out at the field site to minimize transportation costs and to allow any waste products to be returned to the field for their fertilizer value. U.S. Dairy Forage Research Center scientists are developing the juice separations and fractionation process in this joint effort.

Bioremediation of Contaminated Sites

Three methods of bioremediation of contaminated sites involving green plants have been researched and/or proposed:

(1) Treatment with enzymes extracted from transgenic plants as described above.
(2) Treatment with enzymes exuded from the roots of transgenic plants.
(3) Luxury uptake of contaminant by plant species properly matched to the target substance, followed by wet fractionation of the herbage to concentrate the contaminant for disposal or utilization.

If contaminants have leached to depths below the root zones of the plants, pumping of the leachate from wells or tile lines back to the surface would be required for methods 2 and 3.

Forage as a Renewable Energy Source

When combusted, the dry matter in plant material has a heating value of about 7500 BTU/lb. If 30% of the dry matter of a 5 ton/acre crop was removed from the forage by wet fractionation for value-added products, 3.5 ton or 7000 lb of the most fibrous dry matter would be available as an energy source. This fibrous fraction would contain about 52 million BTU which would be the energy equivalent of approximately 375 gallons of oil or two tons of coal (Fig. 10). This material has the potential of being converted to liquid fuel by enzymatic hydrolysis or could be burned directly, possibly after pelleting to improve handling characteristics.

Fig. 10: The most fibrous 70% of a 5 ton/acre alfalfa crop has approximately the same heating value as 375 gallons of oil or two tons of coal.

Intercropping Sorghum into Alfalfa and Reed Canarygrass to Increase Dry Matter Yield

D.R. Buxton and I.C. Anderson

Introduction

In addition to their value as livestock feed, forages could become important for producing biomass for energy conversion. The Department of Energy and the Electrical Power Research Institute (EPRI), which manages technical research and development programs for most of the US electric utility industry, are developing programs to encourage use of biomass from dedicated crops to cofire with coal to reduce sulfur emissions and to reduce the build up of carbon dioxide in the atmosphere. This may give forage producers an additional market for their crops, especially those of low feeding value.

Regardless of the use, forage producers seek to lower the cost of forage production while minimizing adverse environmental effects. One method for reducing production costs is to increase yields so that fixed costs can be applied to more forage. Annual yields of many C_4, warm-season, annual crops are greater than those of traditional C_3, cool-season, perennial forage crops, but annual crops often predispose sloping soils to more erosion than occurs when sod-forming, perennial crops are planted. This research was conducted to determine if annual warm-season forages can be intercropped into perennial, cool-season forage crops to take advantage of the strengths of each crop for improving forage yield and protecting the soil.

Methods

The study was conducted near Ames in central Iowa (1989-1992) and near Chariton in southern Iowa (1989-1990). The Ames site was on a soil with a slope of less than 1% and the Chariton site was on a soil with a slope of 2-7%. 'M-81E' sweet sorghum and 'FFR 201' sorghum x sudangrass were planted in rows 30 inches apart between late May and early June in both sole-crop plantings and when intercropping into 'Arrow' alfalfa or 'Venture' reed canarygrass. The sorghums were not interseeded into alfalfa or reed canarygrass until the year following their establishment. When seeded into reed canarygrass or alfalfa, sorghum planting followed the first harvest of these forage crops. A slot power tiller was used to till a 4-inch-wide strip for each row to a depth of 3 inches before planting. After planting the sorghums and before emergence, paraquat was sprayed over each row in a 6-inch-wide band to slow alfalfa or reed canarygrass growth so that competition to the emerging sorghum plants was reduced.

After the establishment year, sole alfalfa was harvested three times each year and sole reed canarygrass was harvested twice. The intercropped systems were harvested three times each year. The first consisted of only alfalfa or reed canarygrass. The second harvest in mid to late August consisted of sorghum and alfalfa or reed canarygrass. The third harvest taken in early October consisted of regrowth of both species.

Results and Discussion

The two sorghums were equally effective in increasing dry matter yield when intercropped into alfalfa or reed canarygrass (Tables 1 and 2). Intercropping was more successful into alfalfa than into reed canarygrass. Average

biomass production of intercropped alfalfa was 45% greater than that of sole alfalfa at Ames and 28% greater at Chariton. Intercropping sorghum into reed canarygrass at Ames raised biomass production by 34% over sole reed canarygrass. Establishing sorghum in alfalfa or reed canarygrass was most successful when normal to above normal rainfall occurred during the spring. Intercropping sorghum into alfalfa did not have a detrimental effect on stands. Sorghum intercropped into reed canarygrass appeared chlorotic and lacked vigor regardless of the N rate or the amount of rainfall received. The tilled slots remained free of vegetation from year to year making it relatively easy to reestablish the seed bed each year.

Conclusions

Yields of forage can be significantly improved by interseeding sorghum into pure stands of either alfalfa or reed canarygrass and still maintain the protection against soil erosion provided by the perennial species. Success was greater with alfalfa than with reed canarygrass for increasing yield, but reed canarygrass offered more protection against soil erosion.

Table 1. Dry matter yield and neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP) concentrations of biomass grown at Ames.†

Cropping system	Yield	NDF	ADF	CP
	t/ac		%	
Alfalfa	4.64	49.9	39.0	18.8
Reed canarygrass	3.66	63.9	38.7	8.8
Sweet sorghum (SWS)	7.81	56.1	35.6	5.6
Sorghum x sudangrass (SSH)	7.14	54.7	36.1	5.6
Alfalfa/SWS	6.83	52.4	38.5	16.2
Alfalfa/SSH	6.65	54.5	39.0	15.6
Reed canarygrass/SWS	4.87	62.2	37.1	11.2
Reed canarygrass/SSH	4.96	62.5	37.9	11.2
$LSD_{(0.05)}$	0.54	2.4	1.8	0.6

†Data are for a 164 lb N/ac fertilizer rate and averaged over years.

Table 2. Dry matter yield and neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP) concentration of biomass grown at Chariton.†

Cropping system	Yield	NDF	ADF	CP
	t/ac		%	
Alfalfa	4.15	48.3	36.4	17.5
Sweet sorghum (SWS)	8.34	52.7	32.5	6.2
Sorghum x sudangrass (SSH)	7.77	46.2	28.2	8.1
Alfalfa/SWS	5.00	52.5	37.5	16.2
Alfalfa/SSH	5.67	53.1	37.8	15.6
$LSD_{(0.05)}$	0.71	2.3	1.7	0.6

†Data are averaged over 82 and 164 lb N/ac fertilizer N levels and years.

Use of Deeply Rooted Perennial Forages for Subsoil Nitrate Removal

M.P. Russelle, S.D. Evans and D.K. Barnes

Agricultural nitrogen management continues to be plagued by uncertainty. Although N fertilizer recommendations have been improved and can be made relatively site-specific, uncertainty is generated by the dynamic nature of N cycling in the soil and by the overriding impact weather has on the cycle. Nitrogen "losses" (as gases, in runoff, or as solutes in water draining below the root zone) are common to all ecosystems and generally increase with system productivity. It is inevitable that occasional problems will arise in N management, even when conscientious efforts are made to avoid them.

Leaching losses of nitrate-N occur when nitrate availability exceeds crop demand and downward water flow carries the N below the root zone. Improper rates or timing of N applications in fertilizer, animal manure, or crop residues have resulted in large nitrate-N leaching losses. Nitrate leaching below the root zone of annual crops is likely in about one-half the USA due to typical rainfall patterns.

Remediation of nitrate-contaminated subsoils is only possible by crops with sufficiently deep rooting habit. Several perennial species have deeper root systems and use more water during the year than annuals. Forages provide the additional benefit of high growth rates, multiple harvest opportunities, improved soil structure, and an economic product for the farmer. The resulting forage can be fed on-farm or transported to other herds. Alfalfa is a deeply rooted perennial crop that is effective in recovering deep subsoil nitrate in semiarid areas. It is not known how effective alfalfa is in removing soil N under more humid conditions, or how it compares to other deeply rooted perennial forages. We conducted this research to compare the ability of four perennial forages to remove subsoil nitrate.

Materials and Methods

Four forages [effectively nodulated 'Agate' alfalfa; ineffectively nodulated (non-N_2-fixing) Agate alfalfa; a cool season grass, 'Palaton' reed canarygrass; and a warm season grass, 'Forestburg' switchgrass] were seeded in paired plots at Morris, MN in spring, 1990. The plot area had been the site of a long-term manure management study in which various rates of animal manure and fertilizer had been applied for five years, beginning in 1972. Corn had been grown on the plots since 1972, and treatment differences in corn yield had disappeared by 1988. Nitrate accumulations in the upper 3m of soil ranged from about 150 kg/ha in the control plots to about 1750 kg/ha in plots that had received 224 Mg/ha solid beef manure.

Alfalfa was harvested twice in 1990, three times in 1991, and four times in 1992, and the grasses were harvested twice each year, according to recommended practices for the area. Herbage yield and N content were determined. Symbiotic dinitrogen fixation of Agate alfalfa was estimated as the difference in N content of Agate and Ineffective Agate. Soil samples were taken each fall to 3m for nitrate-N analysis, and root length density was determined in fall 1992.

Results and Discussion

All forage crops had higher yields with increasing soil nitrate availability, but increases were smallest in Agate alfalfa. Total herbage yield over the three years ranged from a low of 23.9 Mg/ha to 35.4 Mg/ha for Agate, 14.6 to 33.2 Mg/ha for Ineffective Agate, 9.0 to 19.4 Mg/ha for Palaton reed canarygrass, and 10.7 to 19.4 for Forestburg switchgrass. Forage yield of Ineffective Agate equaled that of effective Agate on plots with high nitrate

concentrations. After the establishment year, there was a close relationship between herbage yield and N removal (Fig. 1). Ineffective Agate alfalfa removed at least 140 kg/ha more N over the three years than reed canarygrass, and 700 kg/ha more N than switchgrass. Soil analyses were too variable to draw conclusions about the depths and amounts of nitrate removal.

Estimated symbiotic dinitrogen fixation declined with increasing nitrate availability, but at progressively smaller rates with increasing stand age (Fig. 2). In each year, there were plots in which dinitrogen fixation appeared to be halted by abundant soil nitrate supply, although we could not confirm this observation with soil nitrate analyses.

Reed canarygrass had the greatest root length density of all species to about 1m. Roots were found under all forages to 2.5m, but switchgrass was the shallowest rooting in most plots. The soil is derived from a dense glacial till, which has dry bulk densities averaging 2.0 Mg/m^3 at 2.5m. Root elongation in many species is restricted by bulk densities above 1.7 Mg/m^3, so the presence of roots below about 1m in this soil was probably limited to cracks or biopores. In many other deep soils of the midwestern USA and Canada, these species should produce more abundant roots than we found below 1.5m.

Conclusion

Large amounts of herbage N can be removed from subsoils that have high nitrate buildup. Alfalfa removed the most N in herbage and, because of its additional high quality as a forage, should be the most economic species for subsoil nitrate remediation.

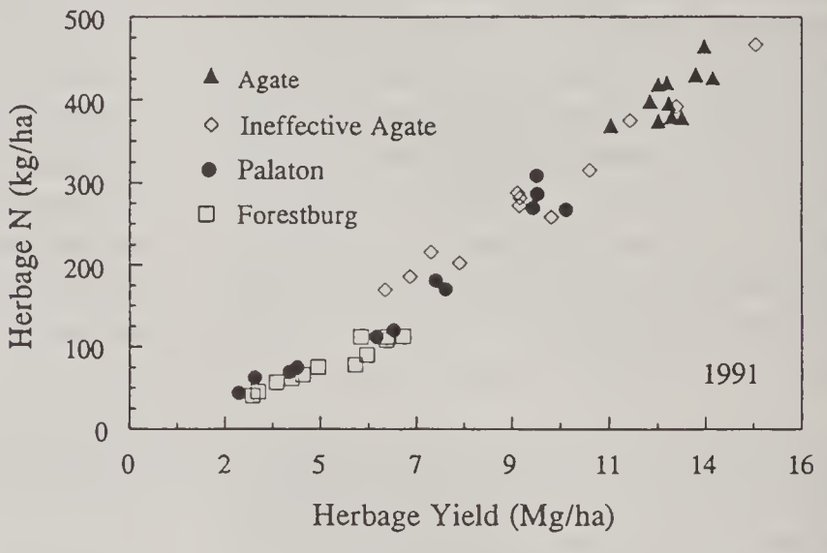


Figure 1. Relationship between herbage dry matter yield and herbage N content for four forages, Agate and Ineffective Agate alfalfa, Palaton reed canarygrass, and Forestburg switchgrass, in the year after stand establishment at Morris, Minnesota.

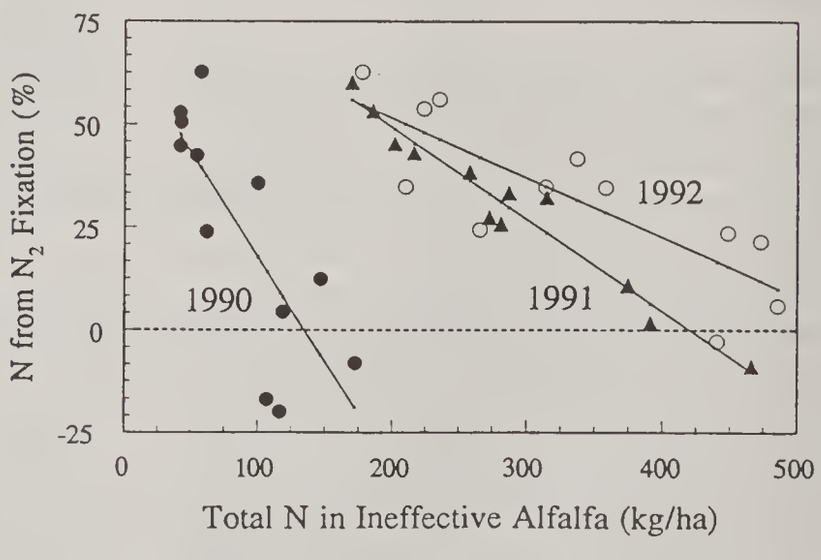


Figure 2. Estimated percentage of herbage N derived from symbiotic dinitrogen fixation in Agate alfalfa in relation to soil nitrate-N availability (as indicated by herbage N content of Ineffective Agate alfalfa) at Morris, Minnesota. Stands were established in spring, 1990.

Crop Sequence Effects on N Response of Corn and Soil Inorganic N

J.A. Lory, M.P. Russelle and G.W. Randall

Fertilizer N and manure frequently are applied to corn grown after alfalfa at rates similar to those applied to continuous corn, although corn following alfalfa typically requires less additional N to attain maximum dry matter yield. In one survey of the 10 major corn producing states in 1987, average fertilizer N applied to corn grown in rotation was 140 kg/ha, compared to 154 kg/ha for continuous corn. This over-application of N to rotationally grown corn may increase soil nitrate-N compared to continuous corn, but there is little information evaluating crop sequence effects on accumulation of soil nitrate from fertilizer N and manure.

Although there have been several studies documenting the altered fertilizer N needs of rotationally grown corn, few have included measurements of N recovery in corn grain and stover or have assessed the impact of excess N on soil nitrate. The objective of this research was to determine the effects of fertilizer and manure N applied in continuous corn vs. corn following alfalfa on corn grain dry matter yield and residual soil nitrate.

Materials and Methods

We conducted two 2-year experiments on a Webster clay loam near Waseca, MN, and on a Tallula silt loam near Rosemount, MN. The first year of each experiment compared the N fertilizer and manure response of first-year corn following alfalfa vs. continuous corn; the second year compared N fertilizer response of second-year corn following alfalfa vs. continuous corn. Blocks of alfalfa and corn were established in 1988 at both locations. Experiment 1 at each location began in 1990; Experiment 2 began in 1991. Three replicates were used at Rosemount and four were used at Waseca.

The alfalfa was spring-plowed in 1990 at both locations and in 1991 at Rosemount but was fall-plowed in 1990 for Exp. 2 at Waseca. In spring of both years, five evenly-spaced rates of fertilizer N as urea were applied and three rates of dairy cow manure slurry were applied and immediately incorporated. Manure N response was not assessed in second-year corn. Corn grain yield was determined at physiological maturity, and soil nitrate-N was measured in fall and spring of each year to a depth of 2.4m.

Results and Discussion

At Rosemount, N applied to first-year corn following alfalfa did not alter grain yield. At Waseca, results were more variable (Fig. 1). Fertilizer N increased grain yield in both sequences, but response was less in first-year corn than continuous corn. Crop sequence had an effect on crop response to manure in Exp. 2, but not in Exp. 1.

There was a greater increase in soil nitrate-N in first-year corn following alfalfa than continuous corn at Rosemount, although the effect of crop sequence was not evident until the following spring (Fig. 2). Application of 157 kg fertilizer N/ha increased soil nitrate by 45 kg N/ha in first-year corn compared to continuous corn. In both crop sequences and both years, most accumulated nitrate-N was below 0.6m by the following spring, and in one year nitrate was apparently leached below the corn root zone (generally limited to 1.5m). At Waseca, soil nitrate-N also was affected more by applied N in rotationally grown corn than continuous corn in Exp. 1, with an 88 kg N/ha increase in soil nitrate-N in the top 1.5m of soil with 157 kg N/ha fertilizer N application vs. no effect on residual nitrate in continuous corn. In Exp. 2, there was little effect

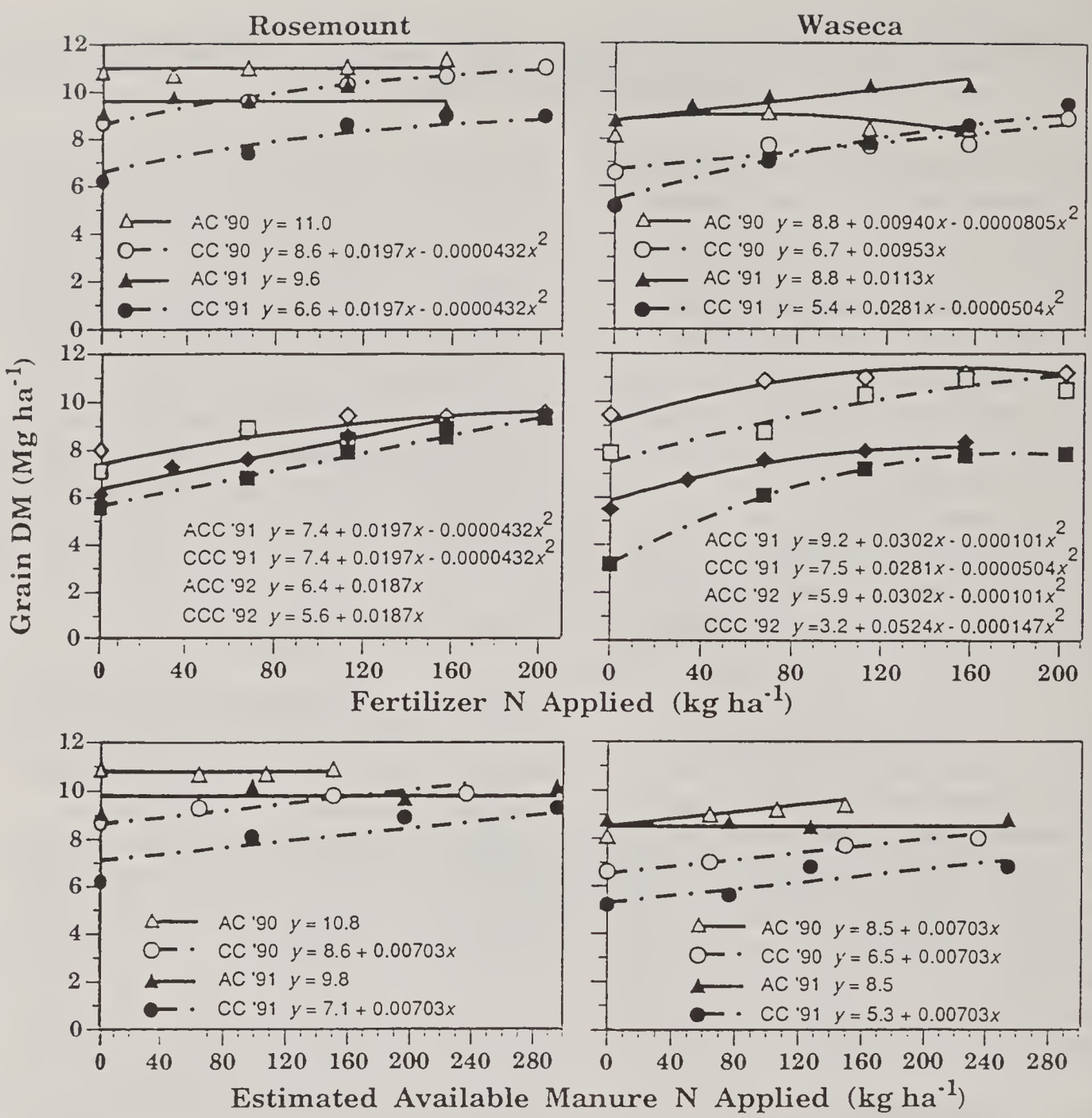


Figure 1. Corn grain dry matter yield response to fertilizer N and estimated available manure N at two Minnesota locations in the indicated years. Top) Response to fertilizer N of first-year corn after alfalfa (AC) vs. continuous corn (CC); Center) response to fertilizer N of second-year corn after alfalfa (ACC) vs. continuous corn; Bottom) response to manure N of first-year corn vs. continuous corn (using Sutton's availability index). Data points are means of three replicates at Rosemount and four at Waseca.

of applied N on soil nitrate because of high precipitation; soil nitrate was presumably lost by leaching or denitrification in that experiment.

Manure provided substantially less N to corn than predicted using Sutton's availability index. Patterns of nitrate distribution in the soil profile were similar for both N sources at both locations (data not shown). The relative impact of agronomically equivalent amounts of N as manure or fertilizer depended on year, crop sequence, and location.

Second-year corn following alfalfa responded like continuous corn at Rosemount but required less N than continuous corn to attain maximum yield at Waseca. Crop sequence

had no effect on residual soil nitrate following second-year corn or continuous corn at either location (data not shown).

Conclusion

This research emphasizes the importance of reducing N applications to first-year corn following alfalfa, a practice already recommended by most states. Our results demonstrate the sensitivity of soil nitrate-N to applied N in alfalfa-corn rotations and suggest that reducing N applications to corn grown after alfalfa will have distinct economic and environmental benefits.

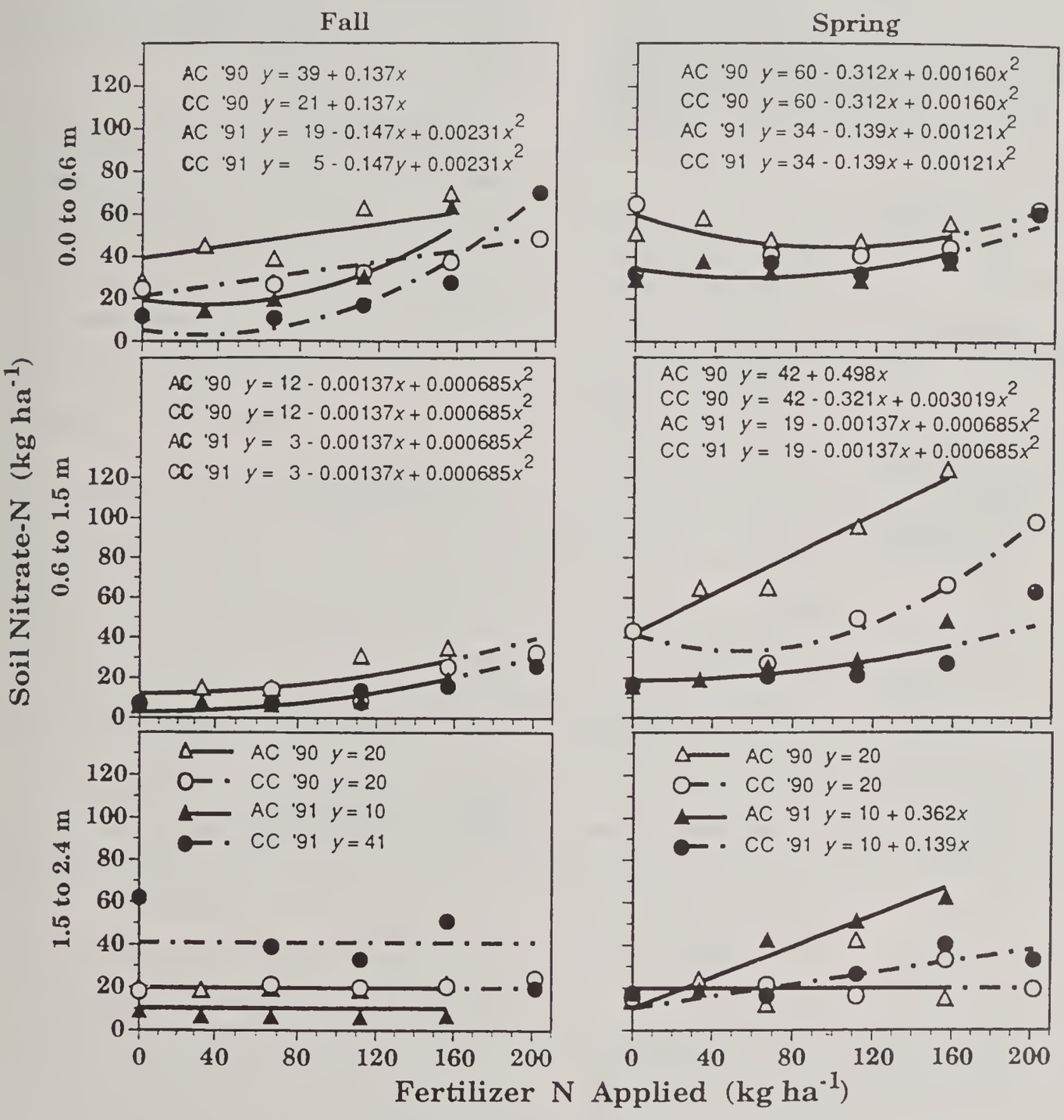


Figure 2. Residual soil nitrate-N amounts in fall and the subsequent spring after first-year corn following alfalfa (AC) and continuous corn (CC) at Rosemount, MN, in the indicated years. Data points are means of three replicates.

A Classification System for Factors Affecting Crop Fertilizer-N Response

J.A. Lory, M.P. Russelle and G.W. Randall

The goal of N fertilizer management is to optimize the relationship between high N fertilizer use efficiency and high yield. This requires adjusting fertilizer management for many factors that affect N fertilizer use efficiency, including environment, soil type, crop sequence, plant variety, and other factors. No framework has been used to define the best strategy to account for a given factor in N fertilizer recommendations. The objective of this work was to construct such a framework and test it with data from field experiments.

Theoretical Framework

The effect of any variable on N fertilizer response of a crop can be classified based on three components: i) y-shift effects; ii) x-shift effects; and iii) interaction effects. These can be understood by viewing N fertilizer response within the larger context of yield response to the available N supply, which includes N derived from fertilizer and other sources, such as soil organic matter (Fig. 1).

A *y-shift effect* alters yield independently of either N supply or N fertilizer (Fig. 1a); that is, it changes intercept coefficients but not slope coefficients. This case might occur in a crop rotation when the previous alternate crop reduces a disease organism compared to continuous culture of the crop being tested. Y-shift effects have no impact on N fertilizer response, so adjustments in N fertilizer recommendations are not needed.

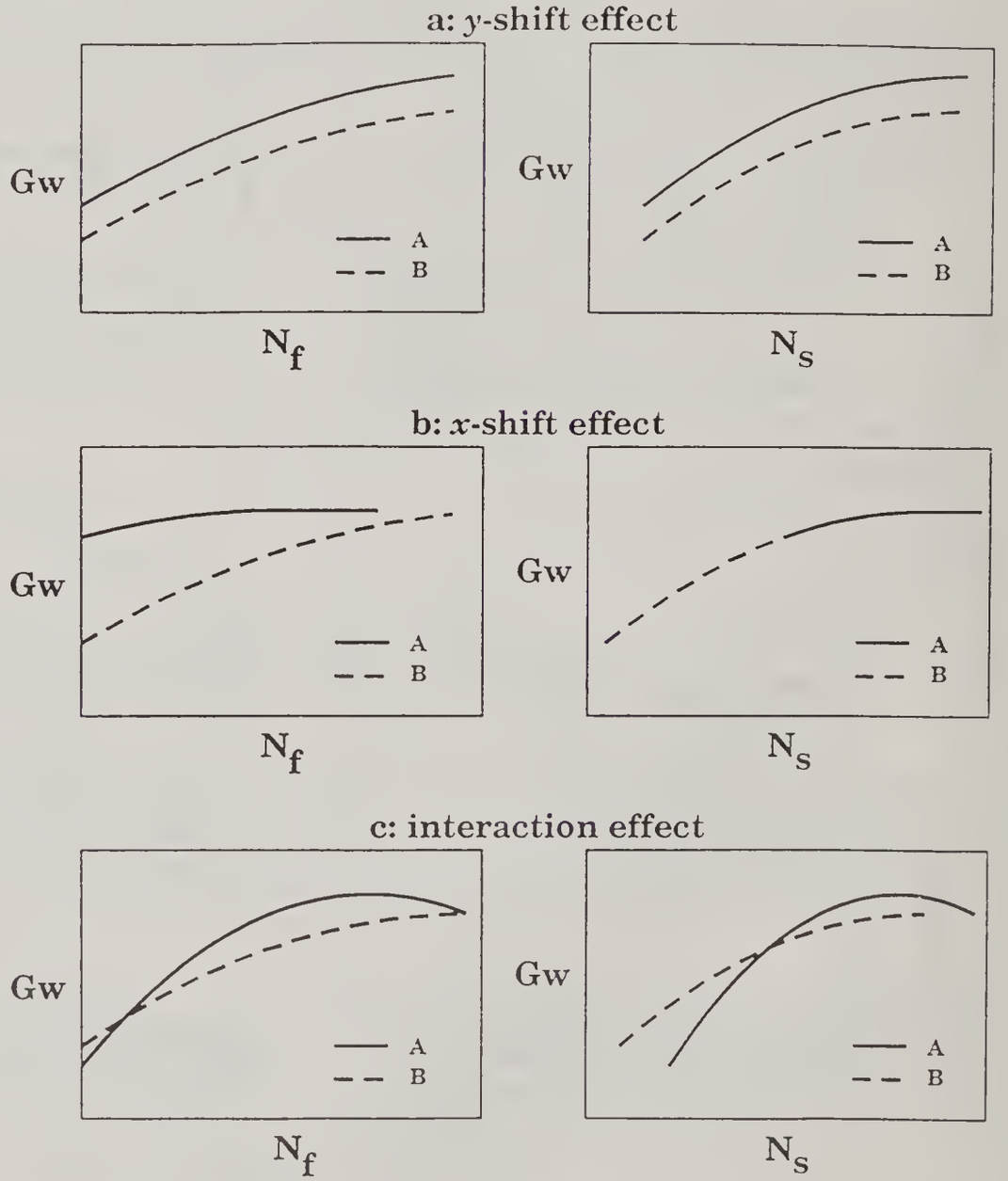


Figure 1. Three potential components of any factor's effect on N fertilizer response. Each is defined by unique relationships of grain yield (Gw) with N fertilizer (Nf) and N supply (Ns).

An *x-shift effect* causes an interaction of yield response to N fertilizer, but not of yield response to N supply. Response curves can be aligned by adjusting for available N (moving them along the x axis; Fig. 1b). This case might occur when previous crops result in differing amounts of residual N in the soil for the current crop. Adjustment for an x-shift effect requires quantification of the factor's effect on the N supply, such as is used in the "N credit" approach in fertilizer recommendations of many states.

Interaction effects are those that cannot be accounted for with y- and x-shift methods. An interaction effect causes a unique relationship between yield and N supply for each factor being considered (Fig. 1c). Interaction effects may require that unique N fertilizer recommendations be developed, such as is done in different geographic regions.

Materials and Methods

We conducted two 2-year experiments on a Webster clay loam near Waseca, MN and on a Tallula silt loam near Rosemount, MN. The first year of each experiment compared the N fertilizer response of first-year corn following alfalfa vs. continuous corn; the second year compared N fertilizer response of second-year corn following alfalfa vs. continuous corn. Blocks of alfalfa and corn were established in 1988 at both locations. Experiment 1 at each location began in 1990; Experiment 2 began in 1991. Three replicates were used at Rosemount and four were used at Waseca.

Five rates of fertilizer N were applied to corn in each year. Grain and stover yields and N contents were measured at physiological maturity. Soil samples were taken to 1.5 m in fall of each year from the control plots (0 kg/ha fertilizer N) for nitrate-N analysis. Total N supply was calculated as the sum of fertilizer N, above ground plant N in the control plots, and soil nitrate-N to 1.5 m in the control plots.

We used regression analysis to determine which N response curves differed and treated replicates separately because N supply in the control plots differed frequently.

Results and Discussion

Location altered the relationship between grain yield and N supply (Fig. 2). Location was classified as an interaction effect because y- and x-shifts failed to account for differences in N fertilizer response. This response is not surprising because of differences in soil type, environment, management (fall vs. spring plowing), etc.

Shift models were able to account for most of the crop sequence and year effects at Rosemount (Fig. 2). First-year corn following alfalfa in 1990 was the only exception; however, limited grain yield response to fertilizer N in this treatment reduced our ability to model the curve. Our results agree with those from other work on similar soils in Iowa, and this suggests that N fertilizer recommendations may be reliably adjusted for crop sequence and year effects on these soils.

Simple y- and x-shift effects did not account for crop sequence and year effects at Waseca (Fig. 2), in contrast to our results at Rosemount and to other work on clay loam soils in Iowa. The nature of the interaction between corn grain yields and N supply varied with year at Waseca. Simple adjustments for N supply (such as universal N credits) may not be reliable on these soils.

Conclusion

The classification system we have introduced can identify conditions in which standard N fertilizer recommendations are reliable, but, more importantly, it identifies conditions in which standard approaches do not work. Finding ways to predict fertilizer N needs on these latter sites is critical to protecting the environment and the farm economy.

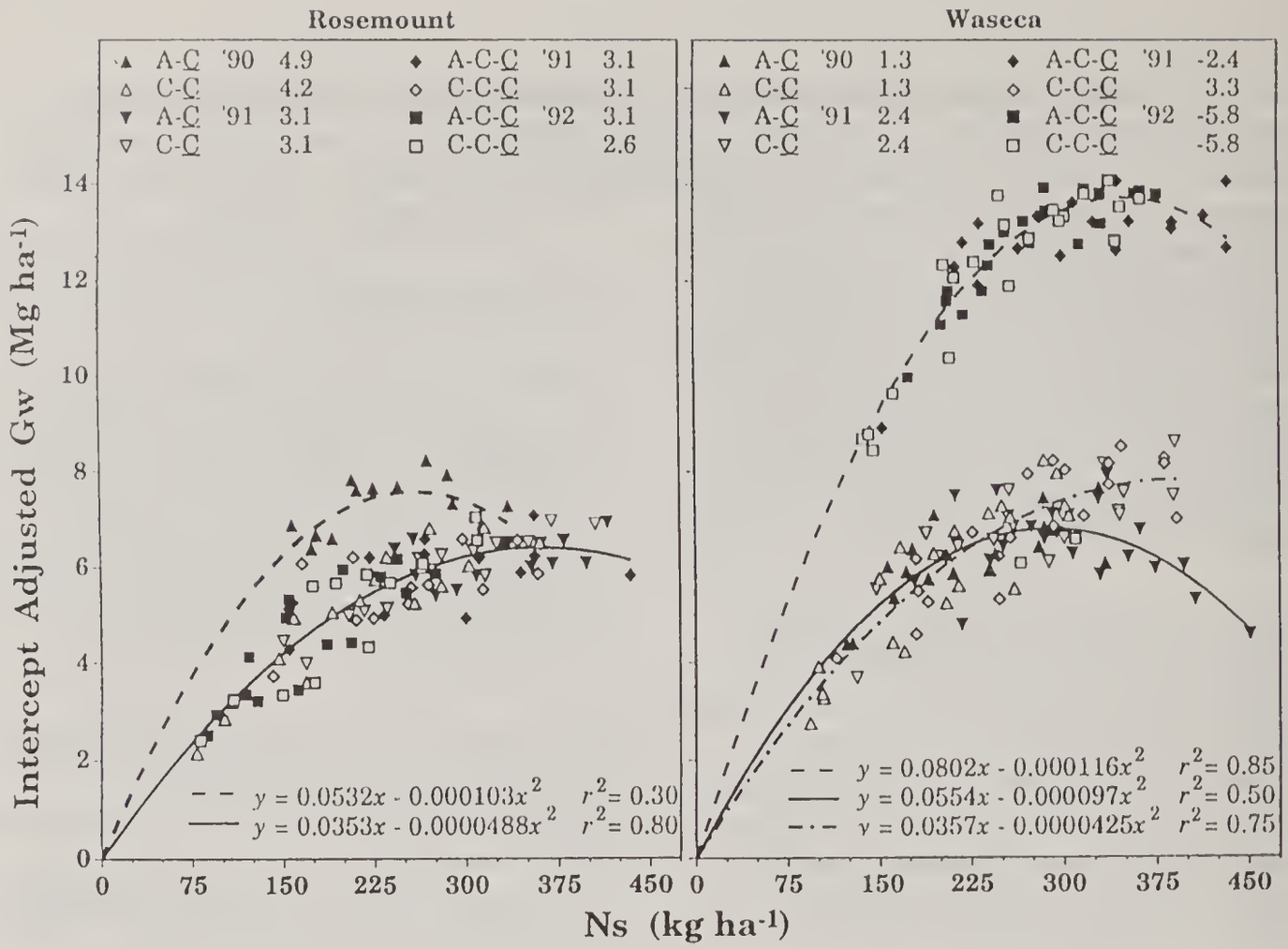


Fig. 2

Figure 2. Relationship between grain dry matter yield (Gw) and N supply (Ns) for corn grown with (solid symbols) or without (open symbols) alfalfa in the crop sequence at two locations in Minnesota over three years. Data are plotted with all intercepts set to zero, with actual intercepts reported in the symbol legend. The underlined letter indicates the position of the corn crop in the crop sequence.

Plant Nutrient Efficiency: A Comparison of Definitions and Suggested Improvement

C.J.P. Gourley, D.L. Allan and M.P. Russelle

Exploiting genetic diversity of plants for enhanced productivity in low fertility soils is a desirable, if not an essential, goal in order to meet food demands for an increasing world population. Diversity among germplasms in the ability to acquire plant nutrients from the environment has been investigated for decades. The term "nutrient efficiency" has been used widely as a measure of the capacity of a plant to acquire and utilize nutrients. Definitions of nutrient efficiency vary greatly, however, and in some cases may be misleading in the quest for increasing productivity and identification of mechanisms for enhanced nutrient acquisition.

Definitions

With regard to yield parameters, nutrient efficiency has been defined as the ability to produce a high plant yield in a soil or other medium that limits production of a standard germplasm. Other definitions include plant production of shoots, or harvestable product, per unit nutrient applied, or "external nutrient requirement", which is the amount of nutrient

required to achieve a given percentage of maximum yield. Yield response per unit added nutrient has been used as a measure of nutrient efficiency.

Alternate definitions emphasize utilization of the absorbed nutrient, e.g., dry mass per unit mass of nutrient, or the reciprocal of nutrient concentration. This "nutrient efficiency ratio" has been used extensively to describe the internal nutrient requirements of plants. Both total and economic product yields have been used in this definition, but there is at least one case in which selection for increased nutrient concentration in a forage to overcome nutrition limitations in livestock resulted in nutrient inefficient plant lines. Others have suggested multiplying the nutrient efficiency ratio by yield to avoid such problems, but the resulting units are odd, dry matter squared per unit nutrient absorbed. Yet other researchers have used "uptake efficiency," defined as the nutrient uptake per unit root length, surface area, or mass.

Screening germplasms for shoot dry mass or harvestable product under nutrient limiting conditions may provide the best estimate of productivity in nutrient-poor soils. However, before a germplasm can be classified as "efficient" or "inefficient" in acquisition and use of a given nutrient, one must identify whether the superior performance under low nutrient conditions resulted from one or more specific mechanisms associated with nutrient accumulation or utilization. Many plant metabolic activities, such as phytohormone production, photosynthetic rate, photoperiodism, and production of ATP, can increase yield potential. A greater overall genetic potential, regardless of the mechanism, is likely to result in higher yields *independent* of nutrient availability.

Recommended Criteria

To reduce the likelihood that differences in nutrient uptake are due to factors other than those mechanisms specifically associated with nutrient acquisition and utilization, it is essential that germplasms achieve similar yields when optimum amounts of the nutrient are available (Fig. 1). Differences in nutrient efficiency then are related to the rates at which the maxima are achieved; therefore well-defined response curves are required for differences to be determined. Under these conditions, two definitions of nutrient efficiency can be used reliably: i) the yield achieved at some low level of nutrient availability, and ii) the external nutrient concentration required to achieve a given percentage of maximum yield. Germplasms that achieve different yield maxima can be regarded only as inferior and superior, not as inefficient and efficient for the given nutrient.

In work reported earlier ("Differences in response to available phosphorus among white clover cultivars", C.J.P. Gourley, D.L. Allan, and M.P. Russelle, in U.S. Dairy Forage Research Center 1991 Research Summaries, p. 7-9), we discussed how the ranking of white clover cultivars varied for various definitions of phosphorus efficiency, and none of the putative mechanisms of improved efficiency were consistently related to cultivar performance. Part of the problem we faced was that most of the cultivars differed in yield potential, thereby confounding the factors involved in nutrient response.

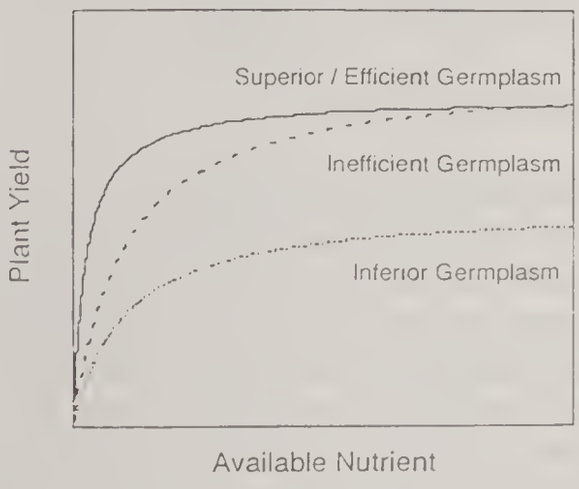


Figure 1. Hypothetical yield response curves of three germplasms differing in nutrient efficiency and yield potential.

Conclusion

It is appropriate to consider mechanisms included in nutrient uptake between different plant germplasms only when the following two criteria are met: 1) equal yield at non-limiting nutrient availability, and 2) where differences exist in the rate at which maximum yields are achieved.

Reaction of Red Clover Germplasm to Inoculation with Ascospores and Mycelium of the Fungus, *Sclerotinia Trifoliorum*

P. Marum, R.R. Smith, C.R. Grau and D.K. Sharpee

Introduction

Sclerotinia root rot caused by *Sclerotinia trifoliorum* is a serious disease in many parts of the U.S. and Europe. In recent years the failure of stand establishment, especially late summer seedings, has been attributed to this organism. Generally, sclerotia in the soil produce spore-bearing apothecia in late summer or early fall and ascospores are discharged and infect leaves and petioles. Development of resistant germplasm has met with little success even though scientists have attempted to select for resistance using mycelium as the source of inoculum. Plants infected by ascospores produce excellent symptoms; however, until recently ascospores have not been used for selection tests because they are not easily produced *in vitro*. The objectives of this study were to develop the appropriate procedures for producing ascospores *in vitro* in the laboratory and to compare the reaction of selected red clover germplasm when inoculated with either ascospores or mycelium.

Materials and Methods

In vitro production of ascospores

Sclerotia were seeded at a depth of 1-2 mm in 8 cm peat pots containing a sterilized mix of soil, sand, and peat (1:1:1; v/v/v) and placed in a flat containing 2 cm of water to keep the sclerotia and soil mix moist at all times. The sclerotia were maintained at 25°C and 12 hr day-length for 28 da to break dormancy. After 28 da, the peat pot, with sclerotia, was placed in a small glass jar with glass top in order to maintain the sclerotia at 100% relative humidity. The sclerotia were maintained at 15°C with 12 hr of approximately 160 u of light. Stipes began to form after 10 -14 da. The asci produced ascospores at about 7-14 da after the stipes formed. Ascospores were released from the asci when the glass lid was removed and were collected on 0.45 um cellulose nitrate membrane filter in a millipore apparatus connected to a vacuum pump. The ascospores were stored on the filter paper at -18°C in parafilm-sealed glass bottles containing silica gel to absorb the moisture.

Growing and inoculating red clover plants

Three-day-old seedlings were planted in plastic trays containing soil mix (see above for soil mix). The plastic trays were then inserted into a second non-draining plastic flat such that the trays could be watered from below with Hoagland's solution. Two-week-old plants were spray-inoculated with ascospores (10,000 spores/ml) or mycelium, depending upon desired test. Inoculum was applied to a "run off" state. Plants were incubated at 15°C at 100% relative humidity in 12 hr day-length for 10-14 da for mycelium inoculations and for 14 da for ascospore inoculations. Plants were evaluated on a scale of 1 = healthy plant, 2 = slight necrosis (few black lesions per plant for ascospores), 3 = moderate necrosis (4-8 black lesions per plant for ascospores), 4 = severe necrosis (more than 8 black lesions per plant for ascospores), and 5 = dead plant. At 28 da after inoculation, the number of surviving plants was determined.

Plant Material and Methods

Four Norwegian cultivars (Molstad, Bjursele, Nordi, and Kolopo-4x), two U.S. cultivars (Arlington and Pennscott), and one experimental strain (C369 — Arlington selected for field tolerance to *Sclerotinia*) were inoculated and evaluated for their reaction to *Sclerotinia trifoliorum*. Two separate sets of tests were conducted - one set using ascospores as the source of inoculum and the second set using mycelium as the source of inoculum. Seven plants were evaluated in each of seven replications for the ascospore inoculations and 21 replications for the mycelium inoculations. Inoculation procedures and evaluation methods outlined above were followed.

Results and Discussion

The reaction of the red clover germplasm to the fungus was consistent whether inoculated with the ascospores or mycelium (Table 1). The Norwegian cultivars, Kolopo(4x) and Bjursele, and the U.S. strain, C369, are the result of previous selection for resistance to the fungus. Both Norwegian cultivars were developed from plants selected as resistant to the mycelium inoculation and C369 was developed from surviving plants from a field test plot area at the Marshfield Experiment Station, Marshfield, WI which had been heavily infested with the fungus. Both Arlington and Pennscott had not been previously selected for resistance and have always given susceptible reactions.

Conclusions

In these artificially produced epiphytotic tests, inoculation of red clover germplasm with either ascospores or mycelium produced similar results. In addition, the results would suggest that some red clover germplasm exists that appears to have a good level of resistance to *Sclerotinia trifoliorum*. However, the mycelium-inoculated derived material has not proven to express resistance under field conditions. The procedures to produce, store, and inoculate with ascospores described in this report will provide an opportunity to select germplasm resistant to ascospores, the natural method of field infection.

Table 1. Reaction of red clover cultivars and strains to *Sclerotinia trifoliorum* using either ascospores or mycelium as inoculum source.

	Source of Inoculum			
	Ascospores		Mycelium	
Cultivar	DSI*	% Surv.**	DSI*	% Surv.**
Kolopo(4x)	3.04	68	3.56	54
Bjursele	3.75	35	3.61	44
C369	3.61	46	3.63	48
Molstad	3.54	46	3.67	45
Nordi	3.42	46	3.82	44
Arlington	3.64	35	3.91	39
Pennscott	3.14	59	3.92	39
Mean	3.45	48	3.73	45
LSD 5%	1.00	21	0.19	10
C.V. (%)	12	17	8.4	35

*Disease Severity Index: 1 = healthy, 5 = dead.

**Percent plants surviving 28 da after inoculation.

Hay Harvest Systems for Illinois: Costs and Value

C.A. Rotz

Introduction

Hay growers are presented with a variety of products and strategies promoted to make better hay. Given the technology available today, what is the best way to make hay? Which products and strategies provide economic return and which do not? These questions were addressed for typical Illinois farms using the computer simulation model, DAFOSYM. The model simulates the growth, harvest, storage and utilization of alfalfa for many years of weather conditions. DAFOSYM provides an excellent tool for making side-by-side comparisons of forage systems in a relatively short time and at a relatively low cost.

Methods

DAFOSYM was used to simulate and compare twelve major harvest options available to midwest hay growers. These systems were modeled on two representative farms for 25 years of Illinois weather. The first farm located in northern Illinois included commercial hay production. Alfalfa was grown on 100 acres of deep silt loam soil. Hay was produced using a three cutting harvest system with an average post harvest yield of 4.6 tons of dry matter (DM) per acre. All hay was sold at a price set proportional to its relative feed value. Prices ranged from $70 to $120/ton DM with an average price of $90/ton DM.

The second farm was a typical dairy farm in southern Illinois. The herd included 100 cows and 76 replacement heifers with an average production of 18,000 lb/cow. About 60% of the herd's forage needs were supplied by 125 acres of alfalfa. The remaining feed came from 125 acres of corn harvested as silage and grain. A four cutting alfalfa harvest system was used where first and fourth were chopped and stored as wilted silage and second and third were harvested as dry hay. Average post-harvest yield on a medium loam soil was 4.8 tons DM/acre. The economic return for the farm was the milk income minus the total cost of feed production and feed supplementation.

Results and Discussion

The DAFOSYM model, in order to compare systems, keeps accurate account of the costs of machinery, fuel, labor and other materials as well as the benefits from reduced losses and improved forage quality. Major options available for hay harvest are compared in Table 1. The absolute values are not as important as the relative changes among systems. The numbers represent the long-term average for typical Illinois farms considering all weather conditions from very favorable to poor.

A mower-conditioner with rubber rolls provides minimal loss and relatively fast drying. Disk mowers increase mowing capacity for a small additional cost. Alfalfa should be laid in wide swaths for rapid drying and raked the morning of the day the hay is baled. Chemical conditioning is an economical method to further speed drying. Swath manipulation with a tedder or inverter may speed drying, but machine costs and losses are greater than the average benefit received. A large bale system can be economical. The cost of a shed is normally justified to reduce storage loss and maintain nutritive value. Hay preservatives such as propionic acid can be used to bale moist hay, but the treatment is not economical unless it is used to prevent major rain damage. A low-cost mow-drying system using a fan to

force ambient air through the hay stack provides effective and economical preservation of moist hay. A new mat drying process provides very rapid drying and exceptionally high quality hay. More research and development of this process is required before it can be used commercially, but its economic potential is good.

Table 1. Effects of several harvest alternatives on field-curing time, hay produced, total production costs and net return over those costs on two types of farms in Illinois.

Harvest alternative	Curing time HQ* (day)	Curing time MQ* (day)	Hay DM HQ* (ton)	Hay DM all hay (ton)	Average relative feed value	Product cost ($/ton)	Net return† ($/acre)
Commercial hay farm (Freeport, IL)							
Base system‡	2.8	5.5	173	392	123	69	84
Rotary disk mower	2.8	5.5	174	392	123	70	76
No conditioning	3.3	7.1	101	302	117	88	5
Chemical conditioning	2.3	5.0	225	403	127	71	88
Tedding only after rain	2.7	5.1	166	384	122	72	68
Tedding of all hay	2.7	4.6	139	369	122	77	47
Narrow swath, no raking	3.1	6.2	155	398	120	64	99
Round baling, stored inside	2.8	5.7	184	384	123	65	98
Baled moist, preservative treated	2.2	4.6	227	401	123	93	-14
Baled moist, mow dried	2.2	4.6	238	418	130	72	93
Mat process with large bales	1.1	1.9	342	456	139	73	120
Dairy farm (Anna, IL)							
Base system‡	2.2	5.0	136	253	131	88	439
Rotary disk mower	2.3	5.0	138	253	132	89	438
No conditioning	2.7	5.5	110	241	127	90	433
Chemical conditioning	1.7	4.5	169	260	136	89	439
Tedding only after rain	2.2	4.7	136	247	131	92	435
Tedding of all hay	2.0	4.1	111	228	130	103	424
Narrow swath, no raking	2.4	5.3	149	271	132	77	455
Round baling, stored inside	2.2	5.1	142	249	132	81	452
Round baling, stored outside	2.2	5.1	124	218	120	95	437
Baled moist, preservative treated	1.8	4.3	172	259	131	103	412
Baled moist, mow dried	1.8	4.3	179	269	138	91	441
Mat process with large bales	0.9	1.9	230	295	146	79	460

* HQ is high quality hay (≤42% neutral detergent fiber) prior to storage; MQ is moderate quality hay (>42% NDF) prior to storage.

† On the commercial hay farm, net return is the value of hay determined as a function of relative feed value minus all production costs divided by the alfalfa crop area. On the dairy farm, net return is the milk income minus the net cost of feeding the herd divided by the total crop area.

‡ Alfalfa harvested as hay is mowed with a 9 ft. mower-conditioner, dried in a wide swath, raked prior to baling, baled in small rectangular bales and stored inside a shed for up to one year. On the dairy farm, only second and third cuttings are harvested as hay; first and fourth cuttings are wilted, chopped and stored in a bunker silo as silage.

An Economic Comparison of Large Round Bale Storage Methods for Dairy Farms

C.A. Rotz, T.M. Harrigan and J.R. Black

Introduction

An increasing number of farmers are using large round bales to reduce the physical labor required in harvesting and handling hay. Also, direct storage costs can be low when bales are stored outside. Unless bales are protected from the weather, though, storage and feeding losses are high. A comprehensive analysis is needed to determine the most economical hay storage methods for dairy farms. Such an analysis must integrate the effects of weather, machinery, labor and other relevant factors on harvest, storage, feeding and use of forages. A simulation model of the dairy forage system (DAFOSYM) was modified and used to compare the long-term performance and economics of five round bale storage methods offering a range of protection and to determine the effect of feeding method on the economic comparison of storage methods.

Methods

Round bale storage methods were compared on a representative dairy farm for 26 years of East Lansing, Michigan weather. Storage methods included shed storage and four methods of outside storage. In the shed, bales were stacked four high in a structure enclosed on three sides. Outside storage methods included: 1) a three layered triangular stack covered with a tarp and set on a well-drained gravel surface, 2) individual bales wrapped in plastic around the circumference and stored on a gravel surface, 3) bales stored on a gravel surface without a cover, and 4) bales stored on the soil without a cover. Feeding options included bales fed ad libitum and bales chopped and fed in a total mixed ration (TMR). For hay fed ad libitum, feeding DM loss was set equal to the average storage DM loss. When chopped and fed in a TMR, the DM loss was 3%.

The farm included 175 acres of alfalfa and 200 acres of corn. First, third and fourth cuttings of alfalfa were harvested as silage with only second cutting baled as dry hay. Hay was baled in 5 ft diameter bales. The relatively small portion of hay produced constituted less than 20% of the forage consumed on the farm. The herd included 150 Holstein cows and 117 replacement heifers with an average milk production above 20,000 lb/cow. At this level, forage quality was the primary constraint to milk production, so higher quality forage provided more milk.

DAFOSYM uses a partial budget format which includes all costs associated with growing, harvesting, storing and feeding the herd. Prices were set to reflect the long-term relative values for the various farm inputs and outputs in 1993 dollars. Hay storage costs for the five storage methods included all additional structures, equipment, labor, fuel and materials required for storage. No cost was assumed for the disposal of used covering materials.

Results and Discussion

A major difference across round bale storage systems was storage and feeding losses. With hay stacked in a shed, average storage loss was 4%. Loss increased with less protection to an average of 12% for unprotected bales set on the soil. On our representative farm, this increase in loss reduced the average annual hay production by 18 tons DM. With feeding loss in hay fed ad libitum set equal to storage loss, the average total loss was 8% in shed stored hay and as much as 24% in uncovered bales set on soil. More alfalfa hay must be purchased to

compensate for that loss. With a high producing herd, milk production may be reduced by the resulting poorer quality hay. On our representative farm with only a small portion of hay in the ration, production was reduced an average of 200 lb/cow when hay was not protected.

Hay storage method has a small effect on machinery, fuel and labor costs with a greater effect on storage cost. When bales were not stacked or wrapped, less machinery time, fuel and labor were required providing a cost reduction of about $600/year. Storage costs were reduced $2,300/year when a storage facility was not used. This cost reduction was partially offset by the increased cost of supplemental feeds. Across all storage methods, the annual cost of feeding the herd varied less than $1,400 or $9 per milking animal and the average annual net return over feed costs varied less than $23/cow (Fig. 1). These were not large differences among the systems, but generally they support the need for good storage facilities. When bales were chopped and mixed in a TMR for feeding, the difference among storage methods decreased. Average annual net return over feed costs varied less than $8/cow among systems with uncovered bales set on the soil providing a net return similar to shed storage (Fig. 1). Covered stacks and uncovered bales set on gravel provided the best economic value, but the advantage was not substantial.

Other factors which affect the economics of hay storage are milk production level, the amount of hay used in the ration and bale diameter. At more moderate production levels, a loss in hay quality can be compensated with supplemental feeds without a drop in production. Since much of the difference among systems is due to differences in milk production, there is less benefit from the investment in storage facilities. When a large portion of the animal's diet comes from hay, hay losses have a much greater impact on supplemental feed requirements and milk production making shed storage and covered stacks more economical than other methods. This effect is influenced by bale size. Smaller (4 ft.) diameter bales have greater loss during outside storage and larger (6 ft.) diameter bales have less loss. Thus, the economic benefit of hay protection is greater for smaller bales.

Conclusion

When a relatively small amount of hay was fed ad libitum to a high producing herd, outside storage in plastic wrapped bales and uncovered bales caused a small decrease in annual net return compared to storage in a shed or tarp covered stacks. When hay was chopped and fed in a total mixed ration, there was little difference among storage methods indicating little value in protecting hay during storage.

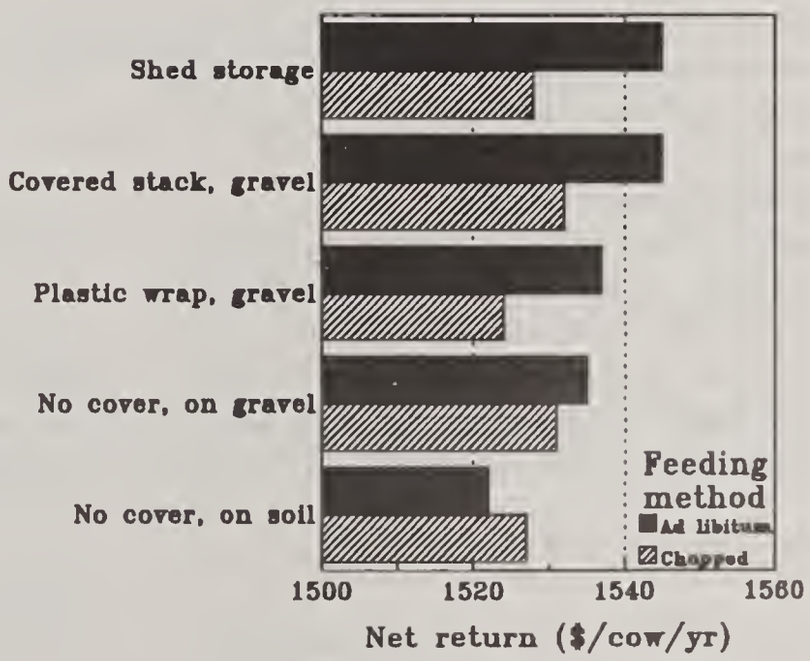


Figure 1. Effects of round bale storage and feeding methods on the net return over feed costs for a 150 cow dairy farm when small amounts of hay are fed in rations along with alfalfa and corn silages.

Mid-size Rectangular Balers: Relative Harvest and Storage Losses

R.L. Huhnke, R.G. Koegel, K.J. Shinners and R.J. Straub

Introduction

Baler configurations have evolved from small rectangular through large round to mid-size (~ 880 x 900 mm) and large (~ 1200 x 1200 mm) rectangular. Each type has certain advantages and disadvantages relative to throughput, harvesting and storage losses, handling and feeding, and transport. Knowledge of these characteristics is important in the selection of baler type.

Since the mid-size and large rectangular balers have come on the market more recently, relatively less is known about their characteristics. Both have bottom-fed bale chambers which have been shown on small rectangular balers to decrease chamber losses relative to side-fed chambers. In addition, the greater throughput, greater bale density, and more compact stacking of these larger rectangular balers make them particularly attractive for some applications, such as off-farm marketing.

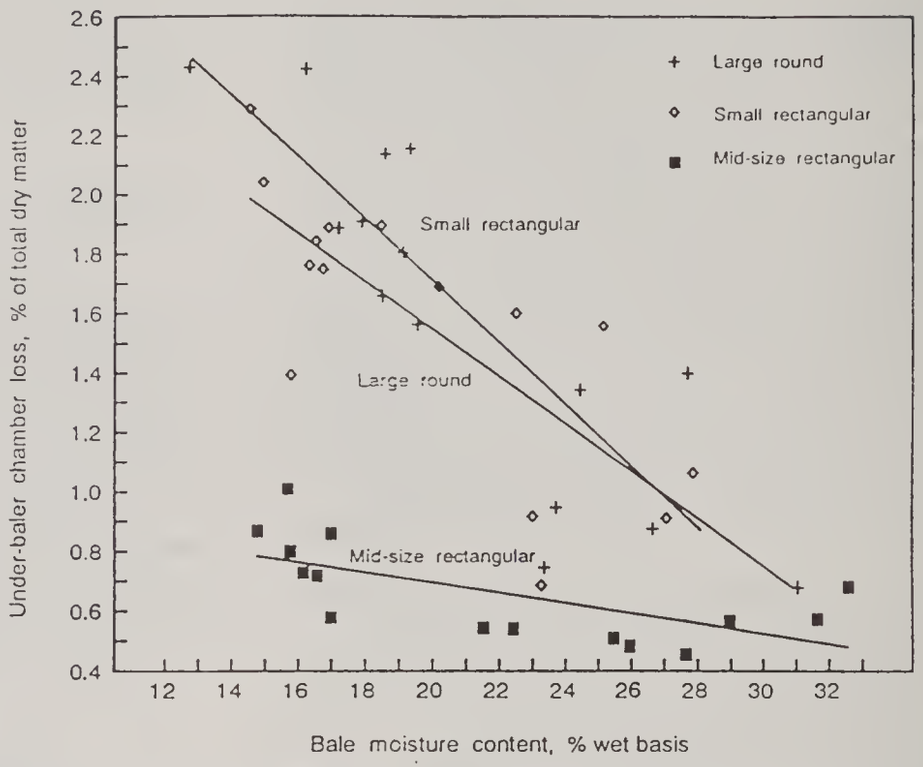


Figure 1. Under-baler losses versus bale moisture content for three baler configurations.

Materials and Methods

Three baler types: small rectangular, large round, and mid-size rectangular were used to bale alfalfa from alternate windrows. Measurements were made of pick-up losses, bale chamber losses, bale moisture, bale densities, and bale temperatures in storage.

Results and Discussion

Bale dry matter density averaged about 50% higher for the mid-size rectangular baler relative to the small rectangular baler (174 vs. 113 kg/m^3).

As shown in Fig. 1, bale chamber losses for the mid-size rectangular baler were considerably less than for the other two types.

Due to their greater density and greater volume:area ration, mid-size bales exhibited higher temperatures in storage than small bales as shown in Fig. 2. The mid-size bales also

averaged higher dry matter losses in storage than the small rectangular bales (6.9% vs. 1.3%). Nutritional analyses of these bales will be reported later.

Conclusions

1. Mid-size bales had approximately 50% higher dry matter density than small bales.
2. Under-baler losses for the mid-size baler were less than half those of the small rectangular or large round balers (0.7% vs. 1.6% vs. 1.6% respectively, of total dry matter).
3. In storage, mid-size bales exhibited higher temperatures and greater dry matter losses than small rectangular bales (6.9% vs. 1.3%).

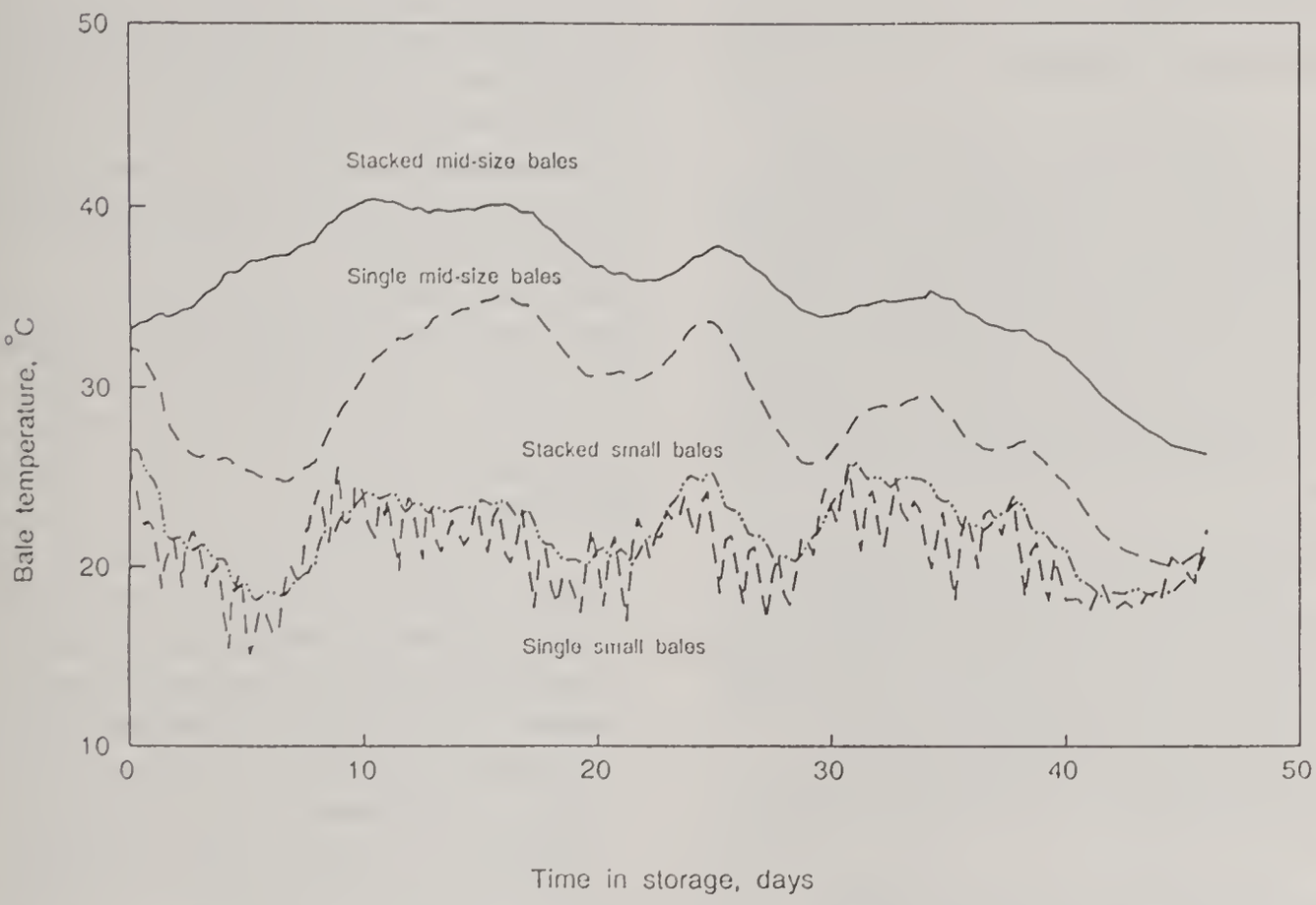


Figure 2. Bale temperature during storage for mid-size and small bales stored in stacks and singly. Average moisture for mid-size bales stored in stacks - 19.0% (w.b.); for mid-size bales stored singly - 18.9T (w.b.); for small bales stored in stacks - 17.9% (w.b.); for small bales stored singly -17.9% (w.b.).

Quick-Drying Forage Mats

R.G. Koegel, T.J. Kraus, R.J. Straub and K.J. Shinners

Introduction

Mats made from alfalfa macerated at the time of mowing and immediately placed on the stubble have been shown in earlier research to dry to a moisture content suitable for baling in less than six hours under favorable conditions. Furthermore, alfalfa so harvested proved to have more rapid and extensive dry matter digestibility (generally 10 - 15% increase) and a greater amount of protein undegraded in the rumen (~ 20% increase) than conventionally harvested material.

Materials and Methods

A "second generation" forage mat machine described in the 1991 and 1992 USDFRC Research Summaries was further modified and evaluated. The direction of rotation of the press drum was reversed, changing it from an "under shot" to an "over shot" configuration. Five 8½ inch diameter rolls were replaced by two 16 inch diameter rolls each made up of 6 pneumatic tires on a common spindle. A flat carrier belt running over the press drum and two small diameter rolls were added to facilitate material handling. This belt provided a receiving location for material coming from the macerator and a location where the belt was sharply kinked to facilitate removal of the intact mat from the belt only a small distance above the stubble.

Mats from the press were evaluated for strength and dry matter bulk density with roll force, press peripheral speed, and number of passes as variables. Feeding of material into the press and release of mats from the press were observed.

Results and Discussion

The modifications to the press appeared to be effective in improving both uniform feeding of forage into the press and releasing intact mats from the press. Some problems with belt tracking were addressed, but require further remediation. For the ranges of roller forces and press speeds used, statistical differences in mat strength and dry matter bulk density were not discernible. Multiple passes through the press did result in increases in dry matter bulk densities of the resulting mats both before and after drying.

In addition to the work carried out at Madison, Wisconsin, prototype machines based on technology developed at Madison have been developed both in Europe and Canada. While the trends are quite comparable to those obtained at Madison, differences in degree of maceration and mat bulk density have led or are expected to lead to some differences in results. One European manufacturer has developed a prototype machine which features maceration or "super-conditioning" but does not press the resulting forage into a mat.

Conclusions

1. Press modification (larger diameter pneumatic rolls and carrier belt) appeared to improve uniformity of feed into the press and release of intact mats onto the stubble.
2. Additional pressure rolls would increase mat dry matter density, but it is not clear whether the increased cost and complexity would be merited.
3. Disadvantages of the flail mower, currently used on the machine, including reduction in fiber length and high power requirement, make its replacement a high priority.
4. Variations beginning to be seen from different groups in degree of maceration and of mat dry matter density necessitate the creation of standardized procedures to determine these parameters. Without specifying these, the value of research on drying rates and nutritional value will be greatly diminished.

Cost Reduction in Forage Harvesting: Upward-Cutting Forage Harvester

M. Stelzle, K.J. Shinners and R.G. Koegel

Introduction

The cost of harvesting and storage of forage crops is a significant part of their overall cost. In addition, where available power limits harvesting rate, timeliness, and hence quality, can be improved by reducing harvester power requirement. It was hypothesized that, by inverting the forage harvester cutterhead, causing it to cut upward through the forage, the kinetic energy imparted to the cut forage particles could be used to throw the forage directly into the trailed wagon. This strategy would eliminate the blower and associated feeding auger or impeller which together consume around 40% of the total required power. In addition, this mechanical simplification would be expected to reduce the acquisition cost of the forage harvester significantly.

Earlier work had shown that the upward cutting configuration could reduce the forage harvester specific energy requirement by about 30%. However, the throw distance was somewhat inferior to that of the conventional cut-and-blow machine. The objective of this work was to determine whether the throwing performance of the upward-cutting harvester could be improved without significant loss of its specific energy advantage.

Materials and Methods

Two methods were used to improve throwing performance: (1) the velocity of air leaving the cutterhead was increased by creating more open area in both the cutterhead housing and cutterhead end walls, and (2) "counter surfaces" perpendicular to and slightly behind each knife cutting edge were used to collect and compress the particles during cutting, so that they could rebound out of the cutterhead as soon as the respective knife had finished cutting through the wad of forage. The distance of the counter surface behind the cutting edge (relief) could be adjusted from 3 mm to 12 mm.

Results and Discussion

Increasing the open area in the ends of the 9-knife cutterhead housing from 0% to 32% increased the delivery spout air velocity from 8.6 m/s to 21.5 m/s and the geometric mean throwing distance from 3.9 m to 5.6 m. Values for the conventional cut-and-blow machine were 22.7 m/s and 6.0 m, respectively.

Effects of the counter surface relief (distance behind the cutting edge) on throwing distance and specific energy requirement are shown in Fig. 1. As the relief was reduced from 12 mm to 3 mm, the throwing distance increased from 7.5 m to 9.1 m while the specific energy increased from 1.2 kWh/t to 1.5 kWh/t.

Table 1 compares the throwing distance and specific energy requirements of three machines: a (downward-cutting) cut-and-throw, a conventional cut-and-blow, and an experimental 12-knife upward cutting machine with counter surfaces set at 9 mm relief. Relative to the conventional cut-and-blow machine, the reduction in specific energy requirement for the experimental machine was about 24%. The geometric mean throwing distance was about one meter less than that of the conventional machine but at 9.3 m (30.5 ft) was considered more than adequate for loading the trailed wagon.

Conclusions

1. Increasing the open area in the ends of the cutterhead and its housing improved the throwing performance of the 9-knife cutterhead by increasing the air velocity.

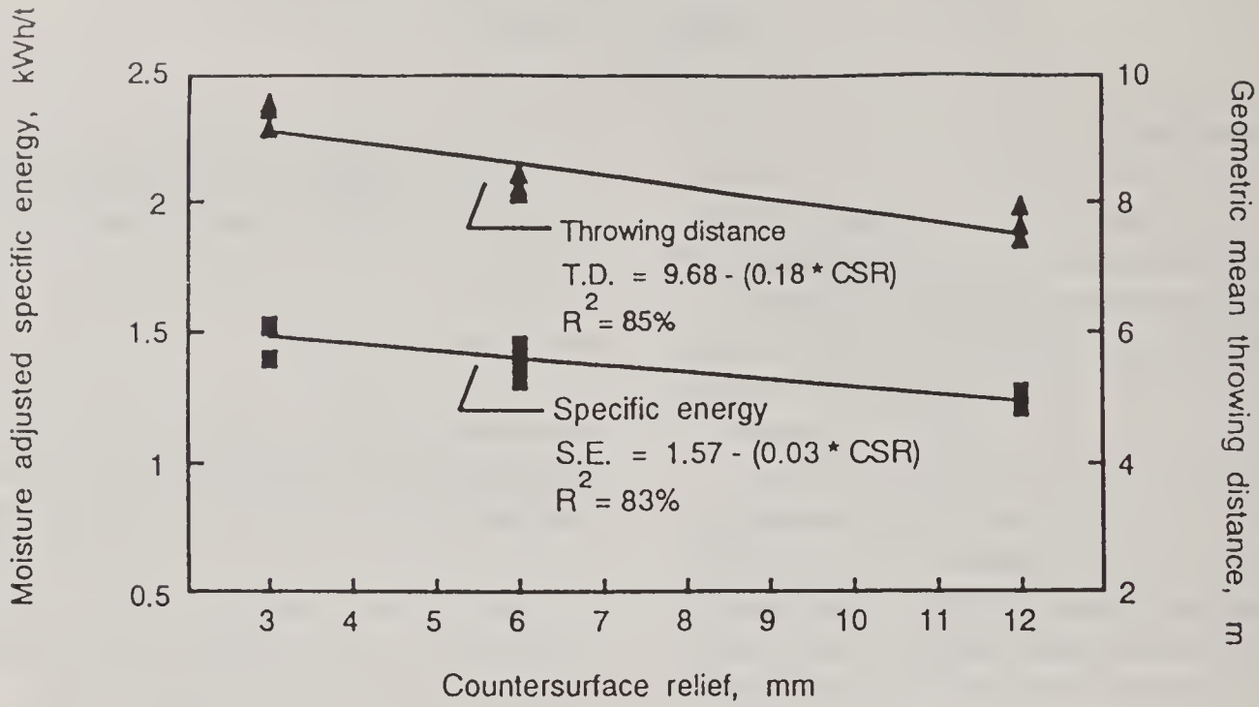


Figure 1. Specific energy requirements and throwing distance with alfalfa as a function of counter surface relief for 9-knife cutterhead.

2. Counter surfaces mounted perpendicular to the knives improved the throwing performance by creating rebound and improving release from the cutterhead.

3. When using 9 mm counter surface reliefs on a 9-knife and on a 12-knife up-cutting cutterhead, throwing distances were 4 and 11% shorter and energy requirements 21 and 24% less, respectively, than those of a conventional cut-and-blow machine for alfalfa.

4. Properly designed up-cutting cutterheads could reduce energy requirements and machine acquisition cost.

Table 1. Throwing distance and specific energy requirements with alfalfa, 12-knife cutterhead with 9 mm relief operating at 1000 rev/min.

Machine Configuration	Moisture content (% w.b.)	Mean length-of-cut (mm)	Moisture adjusted feed rate (t/h)	Geometric mean throwing distance (m)	Moisture adjusted specific energy (kWh/t)	Replicates
Cut-and-throw	59.2	12.1	26.2	8.3	1.63	9
Cut-and-blow	59.6	11.8	26.0	10.4	1.78	9
Exp. upcut	60.7	12.1	25.6	9.3	1.36	9
LSD (P = 0.05)	0.7	0.7	1.2	0.3	0.04	

Fractionation of Alfalfa Juice for Value-Added Products

R.G. Koegel and R.J. Straub

Introduction

Biotechnologists have demonstrated that it is possible to insert genes into green plants, like alfalfa, which will cause them to produce industrially valuable substances not normally produced. Enzymes are the most often mentioned examples of such substances, since it is widely believed that enzymes will be used in unprecedented quantities in the future for a variety of uses. These include: conversion of biomass to liquid fuels, biopulping of wood in the papermaking industry, breakdown of toxic substances in the environment, food processing, cleaning agents, and feed additives. The recovery of enzymes or other valuable substances would be from the plant juice. This involves a series of processing steps which must be carried out at modest temperatures and pH values to avoid inactivation of the enzymes. It is believed that much of the processing must be adaptable to being carried out in mobile units at the field-edge to avoid the cost of transporting lower value fractions and large quantities of water to a central point and that of transporting waste fractions back to the field for their fertilizer value.

The juice contains both particulate and soluble proteins. The particulate matter can be removed by centrifugation after mild warming. The clarified juice contains soluble proteins, sugars and salts. The soluble protein can be concentrated 6-8 fold using an ultrafilter with a 10,000 MW cut-off. The target enzyme would be separated from the soluble protein concentrate by means of an affinity column. The goal of currently involved biotechnologists is to have the target enzyme expressed at a concentration of 1 - 5% of the soluble protein. The remaining soluble protein appears to have potential in the food industry, especially because of its solubility, and when priced comparably to egg white at $3.00 - $5.00 per lb. dry matter would have a market value of $750 - $1250 per acre/year.

The objectives of this research are to evaluate unit processes adaptable to throughputs as high as 25 tons/hr using mobile equipment and to determine the effects of process variables on yields, especially soluble protein.

Materials and Methods

Juice was expressed from alfalfa which had been impact macerated immediately after harvest. Processing variables included: 1) processing temperature: 30 - 45°C, 2) hold time at temperatures, and 3) means of heat addition: water bath, microwave, alternating current through juice, or high voltage pulsed direct current. Particulate matter was removed by centrifugation at 10,000 g for 5 minutes. Soluble protein in the clarified juice was coagulated and filtered. The dry matters in the original juice and all fractions were determined and a mass balance done. A total of 145 separations were carried out during the summer of 1993. Particular interest was focused on the passage of alternating current (AC) through the juice as a means of heat addition, since this was claimed by workers in the former Soviet Union to enhance the yield of soluble protein relative to other means. During the course of the research, the electrodes of the AC treatment apparatus evolved from small stationary stainless steel rectangles to rotating stainless steel disks to rotating carbon disks to stationary, large area (low current density) carbon plates. Steady-state microwave treatment was achieved by pumping the juice through a coil of plastic tubing located in a conventional microwave oven. Flow-rate was adjusted to give the desired outlet temperature with the oven set at full-power.

Workers at U.W. Biotechnology Center monitored the activity of both endogenous and "spiked" enzymes in the various juice fractions on six different occasions throughout the summer.

Results and Discussion

Yields of soluble protein coagulum as a percent of plant dry matter were greater from immature plants (~ 3%) than from more mature plants (~ 2%). Yields generally appeared to decrease as elapsed time between maceration and separation increased. Yields of soluble protein following the AC heat addition were not consistently greater than for other treatments throughout the summer. After incorporation of the large, low current density (.25 amps/cm^2) carbon electrodes, however, the AC treatment gave greater yields in seven out of eight direct comparisons. The average increase in yield relative to microwave heat addition for the final five comparisons of the season was 6% with a range of 2 to 10%. Since seasonal effects can't be ruled out, however, additional verification is needed to determine if there is a consistent yield advantage.

On those occasions when the level of activity of selected enzymes was assayed at various steps of the separation process, no significant problem with loss of activity was observed. The maximum temperature allowable to avoid loss of activity varies greatly from one enzyme to another. Since higher temperatures accelerate particle aggregation which, in turn, facilitates centrifugation, the highest temperature consistent with the target enzyme should be used. No higher than 50°C should be used, however, if a maximum soluble protein yield is desired since, above this temperature, soluble protein begins to precipitate along with the particulate material.

No ultrafiltration was carried out to concentrate the soluble protein in the clarified juice during 1993. However, when this was done on several occasions during 1992, no problems were encountered with membrane fouling as has frequently been reported for whole juice.

Quantities of products resulting from alfalfa fractionation can vary considerably depending on crop maturity and processing parameters.

Overall, approximately 20% of the plant dry matter was expressed in the juice. The dry matter in the soluble protein concentrate and the particulate protein concentrate was approximately 15% and 45%, respectively, of the juice dry matter. Since the protein concentration of the soluble fraction is approximately double that of the particulate fraction (90% vs. 45% according to the literature), the actual protein in the soluble fraction is about 67% of that in the particulate fraction.

Conclusions

1. A series of unit operations has been established for obtaining a soluble protein concentrate from alfalfa.
2. In the case of transgenic alfalfa, a target enzyme with its activity unimpaired could be recovered from this concentrate using established technology.
3. Based on the price of existing food ingredients, the soluble protein fraction (without any enzyme recovery), while constituting only 2 - 3% of the crop dry matter, appears to have a potential market value several times that of the entire crop used as conventional forage.
4. The overall separation process appears amenable to scale-up to throughputs as high as 25 tons/hr in mobile processing units.
5. Information on the functional properties (solubility, foaming, emulsification) of the soluble protein fraction would be required to determine its most appropriate applications in food products and its consequent market value.
6. Profitability of enzyme recovery will depend on the ability of biotechnologists to produce the target enzyme at concentrations of at least 1% of the soluble protein.

Effect of Unloader on Aerobic Activity at the Face of Bunker Silos

R.E. Muck and R.L. Huhnke

Introduction

Bunker silos in the United States are typically unloaded with a front-mounted bucket attached to a skid-steer loader, tractor or payloader. This creates a rough silage face. In contrast, European farmers generally use a specialized silo unloader which produces a smoother surface. Few data are available to indicate whether these specialized unloaders reduce air penetration and aerobic microbial deterioration enough to justify their purchase. If economic benefits were sufficient, U.S. manufacturers would have an incentive for developing equipment for this market.

Methods

Three bunker silos (two containing corn silage, one with alfalfa silage) were sampled in a similar manner. Each silo was unloaded with two types of unloaders: a skid-steer loader and a tractor-mounted silage cutter that milled a smooth face. Each silo face was split into two halves with one unloader randomly assigned to a side. Silage removal each day alternated between the two sides. Each side was sampled twice, with at least one week between samplings. Then the unloaders were switched to the opposite sides, and again each side was sampled twice.

Sampling of a side was performed approximately 24 h after silage had been removed from that side. First, a profile of the silage face was made. At three distances from the side wall, a plumb line was dropped from the top of the silo. Distances between the silage face and the plumb line were recorded at 5 or 10 cm intervals. In the process of taking each profile, four locations for gas sampling were marked: 0.5 m from the floor, approximately 0.5 m from the top and two intermediate locations. Second, gas samples and temperature measurements were taken at four depths from the face (12.5, 25, 50 and 100 cm) at each of the 12 locations. Gas samples were analyzed for O_2, N_2, H_2 and CO_2. Third, after the second sampling of a side, core samples adjacent to the 12 gas sampling locations were taken at two depths (0-12.5 cm, 12.5-25 cm) for analysis of silage density, moisture, pH, fermentation products, and microorganisms (yeasts, molds, acetic acid bacteria, bacillus spores).

Results and Discussion

Based on the profiles, the surface area of the cutter side was 4-9% greater than that of a vertical wall whereas the skid-steer side was 12-37% greater. The differences in surface area between the two unloaders were affected by crop. In the corn silos, the skid-steer loader produced on average a 9% increase in surface area relative to the cutter whereas the increase for alfalfa was 26%. This discrepancy appeared to be caused by the presence of longer forage particles in the alfalfa silage, which could support larger ledges and deeper crevices.

Oxygen profiles within the silos were affected by height, depth and unloader. Oxygen levels were highest at the face and decreased with depth (Fig. 1) as would be expected. In both the alfalfa and the corn silage silos sampled in winter, oxygen concentrations increased with distance from the silo floor (Fig. 2). This appeared to be due to two factors: (i) decreasing density as height from the floor increased, and (ii) typically 50-100 cm at the top edge of the silo was not covered with plastic, permitting vertical and horizontal movement of air. This trend was not significant in the corn silo sampled in summer, presumably due to active

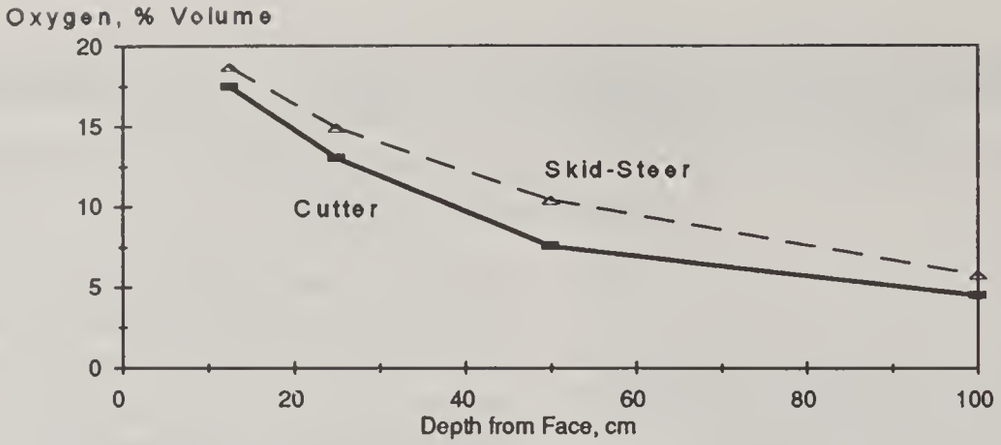


Figure 1. Average oxygen profiles in the corn silo sampled in winter as a function of depth.

aerobic deterioration at the top of the silo and a more uniform silage density with height. The magnitude of the differences between the two unloaders was not large. On average, the oxygen concentration on the skid-steer side was 1.8, 1.1 and 2.2% volume units higher than the cutter side for the winter corn silo, summer corn silo and alfalfa silo, respectively. However, at the 100 cm depth, there were no significant differences between unloaders.

Temperature was only slightly affected by unloader in all three silos. The unloaders had no significant effect on temperature in the alfalfa silo. The skid-steer side was on average 0.9 and 1.5°C warmer on the skid-steer side in the winter-and summer-sampled corn silos, respectively.

Both wet and dry densities were numerically higher (1-8% and 2-6%, respectively) using the cutter in all three silos. However, statistical significance ($p<0.05$) only occurred in the wet densities of the summer corn silo and the alfalfa silo and the dry density of the summer corn silo. Densities at the face (0-12.5 cm) were consistently lower (3-10% and 3-9% for wet and dry densities, respectively) than densities deeper from the face, but statistical significance only occurred in the corn silos.

Silage pH was unaffected by unloader in all three silos and ranged from 3.6 to 3.9 for the corn silage and from 4.6 to 5.0 for the alfalfa. Aerobic microbial populations were low in all three silos. Bacillus spore counts were generally 10^3 to 10^4 colony forming units/g silage. Counts of yeasts, molds and acetic acid bacteria were typically one or two orders of magnitude less than this range. Consequently, there did not appear to be appreciable aerobic deterioration occurring at any of the sampling locations. Counts of yeasts and acetic acid bacteria were consistently higher for the skid-steer side but not always statistically different. These results suggested a lower bunk life for the skid-steer unloaded silage.

Conclusions

The skid-steer loader increased the movement of oxygen into silage at the face of the bunker silo, decreased silage densities and increased aerobic microbial populations. However, based on microbial counts and pH, no significant differences in aerobic losses between the two unloaders were apparent. This would suggest that specialized silo unloaders may not be justified, but further research investigating a wider variety of conditions is needed to confirm these results.

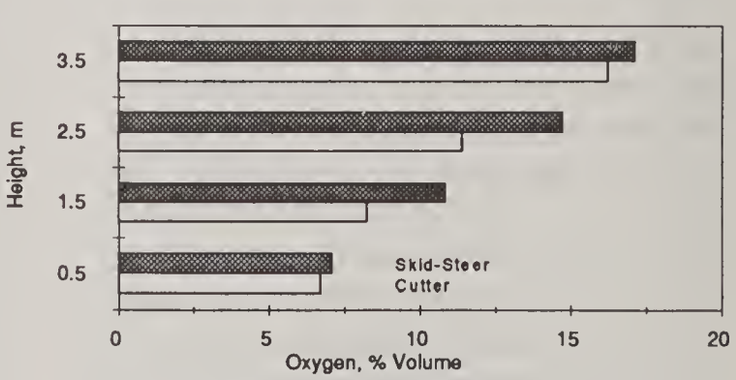


Figure 2. Average oxygen profiles in the corn silo sampled in winter as a function of height.

Alternatives for Covering Bunker Silos

R.E. Muck

Introduction

Bunker or horizontal silos are normally covered with polyethylene plastic that is held in place with used tires. This is necessary to reduce dry matter losses of approximately 40% that occur in the top 50 cm without a cover. Unfortunately, many farmers feel that covering and uncovering involves too much time. Also disposal of the plastic is a problem in some areas of the country. The purpose of this study was to investigate several potential alternatives to polyethylene plastic.

Methods

Chopped whole plant corn (34% DM) was ensiled in quart glass canning jars. Eight different sealing treatments were compared: black plastic, 7.6 cm barn lime wetted with water at 0.12 g/g lime, and six combinations of lime with corn zein films (two film formulations; 3 placements - on top of 3.8 cm dry lime, on top of wetted lime, or sandwiched between a wetted 1.9 cm lime layer and a dry 1.9 cm lime layer). Due to differences in the thickness of the cover, the amount of corn ensiled per silo varied with treatment. Silos covered with plastic contained 510 g corn, the lime treatment - 324 g and the zein treatments - 432 g. Four silos per treatment were ensiled. Two replicates were stored inside at room temperature while two replicates were stored outside. Silos were weighed at 0, 1, 2, 3, 7, 14, 21, 28, 35, 42, 59 and 84 d of ensiling. Notes regarding the visible development of microorganisms were recorded at each weighing.

Results and Discussion

Weight losses from the silos stored inside at room temperature are shown in Fig. 1. All treatments in which lime was wetted experienced much more rapid weight loss in the first 3 to 7 d of the experiment than other treatments. This accelerated weight loss was presumably due to water evaporation from the lime layer. After 14 d, weight loss from the silos was relatively linear with time. Rates of weight loss were statistically similar for 6 of the 8 treatments (1.31-1.45 g/d/silo). The loss rate from the plastic covered silos was significantly slower (0.026 g/d/silo) than that for the other treatments whereas zein formula 1 on the wetted lime had the highest rate of loss (1.66 g/d/silo). After 84 d, only the plastic-covered treatment was free of visible yeast and mold growth. In the other treatments at 84 d, yeast and/or mold growth was observed throughout the silos with the exception of the layered treatment with zein formula 1. In that treatment, mold growth covered only the top two-thirds of the silage. Overall, because the zein treatments had half the lime and one-third more corn per silo, the zein treatments reduced losses by comparison with the wetted lime treatment. However, even the best alternative treatment lost weight at more than 50 times the rate of the plastic-covered treatment.

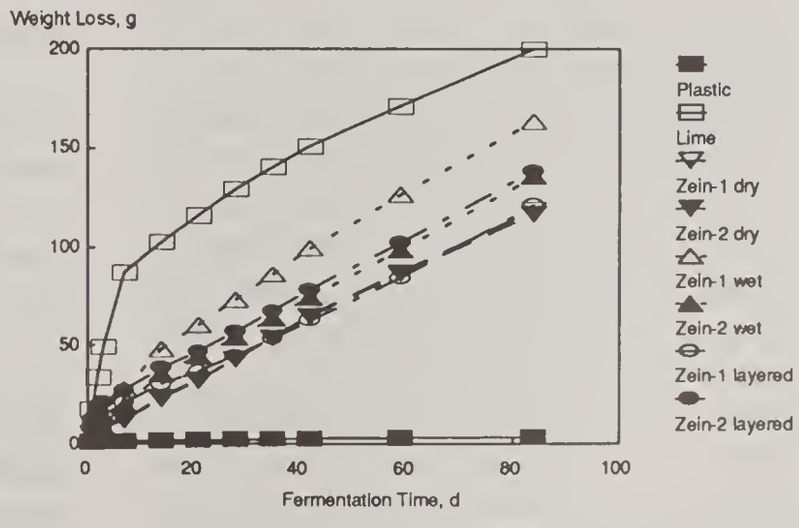


Figure. 1. Weight loss from corn silage stored indoors at room temperature as affected by silo covering.

Losses from the silos stored outside were more difficult to assess. Sampling was halted after 59 d. All treatments with lime had a net gain in weight due to absorption of precipitation. The lime treatment gained 190 g whereas the zein treatments gained between 239 and 313 g. Because of cooler outdoor temperatures, deterioration was not as evident in these silos. However, only in the plastic-covered silos and in the zein treatments applied to the top of the wetted lime were all replicates free of visible yeast and mold growth. The zein treatments on top of dry lime and formula 2 layered between lime had consistent evidence of yeast growth. The pHs of these three treatments at 59 d were also elevated (all 5.39 or higher). The average pH values for the plastic, lime, zein-1 wet, zein-2 wet, and zein-1 layered treatments were 3.87, 4.40, 4.36, 4.16 and 4.76, respectively. Therefore, even though there was no visible deterioration in the zein treatments applied to wetted lime, the elevated pH values relative to the plastic treatment suggested that more deterioration had occurred in these treatments than in the plastic-covered treatment.

Conclusions

None of the alternative coverings compared favorably with black polyethylene plastic. The zein films did reduce losses under indoor conditions relative to lime alone, but losses were still more than 50 times that of plastic. Under outdoor conditions, the zein films applied to wetted lime performed the best of the alternative coverings. However, these coverings absorbed a considerable amount of precipitation as well as permitted aerobic deterioration. Consequently, none of the alternative coverings appeared to be a suitable replacement for polyethylene.

A Comparison of Tower and Bunker Silos on Wisconsin Dairy Farms

C.A. Rotz

Introduction

Silage is a popular method for preserving alfalfa on dairy farms in Wisconsin. A major decision in designing a silage system is the type of storage structure. Primary options include bottom-unloaded sealed tower silos, top-unloaded stave tower silos and horizontal or bunker silos. Costs, labor requirements, energy requirements and losses are all influenced by the type of structure used. Proper comparison and selection of silage systems require a comprehensive analysis. A computer model called DAFOSYM simulates the growth, harvest, storage, feeding and use of alfalfa and corn on dairy farms. By simulating various silage systems for the same farm, the effect of system changes on farm performance and economics can be determined. DAFOSYM was used to compare bunker, stave and sealed silo systems on typical Wisconsin dairy farms.

Methods

DAFOSYM parameters were set to describe small- and large-sized farms. The small farm consisted of 75 acres each of alfalfa and corn used to feed 60 milking cows and their young stock. The large farm used 300 acres of alfalfa and 100 acres of corn to meet the forage needs of 250 cows plus young stock. A four cutting alfalfa harvest system was used on both farms. On the small farm, first and fourth cuttings were chopped and stored as silage with second and third cuttings baled in small, rectangular

bales. Enough forage and grain were raised to meet the needs of the herd with 40% of the forage provided by corn silage. On the large farm, all alfalfa was produced as silage and all corn grain was purchased. Harvest moistures for the top-unloaded stave silos, bottom unloaded sealed silos and bunker silos were less than 65, 60 and 68%, respectively. The dairy herds included milking animals and young stock with 30% of the milking animals in their first lactation. The average annual milk production of the herds was set at 20,000 lb/cow. Prices for milk, excess feeds sold and various farm inputs were set to reflect long-term average values relative to each other. All systems were simulated for 26 years of weather for Madison, Wisconsin.

Results and Discussion

On the small farm, silo type had a small effect on farm performance with a greater effect on farm economics (Table 1). Compared to the stave silo, the sealed silo produced slightly more alfalfa silage. Forage energy content was better preserved in the sealed silo allowing slightly less corn in the ration. The higher equipment cost for the sealed silo was due to the greater initial cost and greater maintenance cost of the silo unloader. The high initial cost of the sealed silo increased storage cost. These greater costs led to a $3,000/year decrease in the net return over feed cost.

The combined effects of lower harvest loss and greater storage loss for the bunker silo system on the small farm provided a slight gain in silage production. This increase reduced the requirement for grain and purchased hay. Greater breakdown of true protein to non protein nitrogen in the bunker created a greater need for protein supplementation. Since a silo unloader was not used, equipment costs were lower for the bunker silo. The cost of constructing small bunkers was greater than the initial cost of stave silos which increased storage costs. Labor costs were a little greater due to the need for an extra person to operate the packing tractor during filling. With all totaled, the increased costs led to a decrease in net return for the farm of about $1,000/year.

On the larger farm, the benefits among silo types shifted more toward bunkers. Greater loss in bunker silos led to lower silage production and the need for more corn in animal rations (Table 1). Equipment cost was again less due to faster filling of bunker silos and the elimination of the silo unloader. The electrical requirement also dropped when a silo unloader was not used. When two bunker silos of similar size to the tower silos were used, the storage cost was similar. By going to one large bunker without walls on either end, the storage cost was reduced by $3,000/year. This large bunker increased the net return by $2,000/year.

Plastic covers are often not used on bunker silos. This reduces the cost for plastic and installation labor and eliminates the inconvenience of dealing with the plastic and tire weights. Without a cover, storage cost was about $4,000/year less for the bunker system, but purchased feed cost was $12,000/year greater. Along with a small drop in milk income, the net return to the farm was $6,500/year less compared to the use of stave silos.

Conclusion

On a small Wisconsin dairy farm, top-unloaded stave silos provided the most economical method of storing alfalfa silage. On a large farm, the use of two bunker silos provided a net return similar to two stave silos. Use of one large bunker improved the net return by a small amount. When a cover was not used on the large bunker, stave silos were most economical.

Table 1. Effect of silo type on feed use and costs for small and large dairy farms in Wisconsin.

Production or cost parameter	Unit	Small farm Tower, stave	Small farm Tower, sealed	Small farm Bunker	Large farm Towers, 2 stave	Large farm Bunkers, two	Large farm Bunker, one
Alfalfa hay production	ton DM	177	175	176	—	—	—
Alfalfa silage production	ton DM	104	107	107	1247	1207	1197
Corn silage production	ton DM	181	181	181	672	672	672
Corn grain production	ton DM	139	139	139	—	—	—
Corn grain purchased (sold)	ton DM	(21)	(24)	(23)	318	371	377
Alfalfa purchased (sold)	ton DM	2	3	4	30	31	34
Soybean meal purchased	ton DM	23	24	26	166	164	166
Field and feeding equipment cost	$	25,224	26,532	24,329	47,589	42,317	42,310
Fuel and electric cost	$	2,366	2,372	2,331	9,188	8,795	8,790
Feed storage cost	$	6,478	8,303	7,393	17,973	21,202	17,678
Labor cost	$	10,151	10,040	10,648	17,606	17,763	17,766
Net of purchased and sold feeds	$	3,400	3,241	3,654	74,363	79,660	81,072
Total feed cost	$	66,620	69,489	67,357	205,088	208,306	206,184
Milk income	$	147,499	147,499	147,499	614,579	614,579	614,579
Net return over feed costs	$	80,879	78,010	80,142	409,491	406,273	408,395
Feed cost / unit milk	$/cwt	5.55	5.79	5.61	4.10	4.17	4.12

Enzyme, Inoculant, and Formic Acid Effects on Cell-Wall Concentration and Digestibility of Silage

E.M.G. Thorstensson, D.R. Buxton, J.R. Russell and J.W. Young

Introduction

Forage cell-wall concentration, which increases with maturity, limits ruminant dry matter (DM) intake and digestibility of forage plants. Because forages are important components of ruminant rations, reduction of the cell-wall concentration is desirable. There is interest in the use of cell-wall degrading enzymes, bacterial inoculants, and formic acid to improve silage quality. Cell-wall degrading enzymes can decrease cell-wall concentration and increase cell solubles resulting in higher potential intake and digestibility of ensiled forages. When bacterial numbers are limiting, bacterial inoculants often enhance silage fermentation. Formic acid restricts fermentation and maintains water-soluble carbohydrate concentration that is normally used during fermentation.

The objectives of this study were to determine (1) the effects of cellulase, pectinase, bacterial inoculant, and formic acid on cell-wall concentration and DM digestibility of orchardgrass and alfalfa silages harvested at different maturities and (2) the impact of decreased cell-wall concentration on DM digestibility of the silages.

Materials and Methods

Orchardgrass and alfalfa were harvested at three maturities with 2-week intervals within May-June and July-August growth cycles. Herbage was wilted to 29-33% DM and

ensiled for 60 d in 900 g glass jars. Cellulase was applied alone to the forage or in combination with pectinase, inoculant, or formic acid. Application rates were 2, 10, and 20 mL/kg forage of cellulase, 0.3 and 3 μL/kg forage of pectinase, 10^5 colony forming units of a mixture of *Lactobacillus plantarum* and *Pediococcus cerevicae*/g forage of the inoculum, and 4 mL/kg forage of formic acid. The cellulase (Multifect CL) was derived from *Trichoderma longibrachiatum* and had a minimum carboxymethyl cellulose activity of 2500 IU/mL. The pectinase (Cytolase PCL1) was produced from *Aspergillus niger* and had a minimum activity of 1300 apple pomace pectin viscosity units/mL. Both enzymes were supplied by Genencor International, Rolling Meadows, IL, and the inoculant (Biomate SI) was supplied by Chr. Hansen's Laboratory, Inc., Milwaukee, WI.

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were sequentially analyzed on fresh herbage and silage samples. Hemicellulose concentration was calculated as the difference between NDF and ADF concentrations, and cellulose concentration was calculated as the difference between ADF and the sum of ADL-plus-ash concentrations. Also, in vitro DM digestibility (IVDMD) analysis was conducted. The experimental design was a split, split plot with species as the whole plot, time of harvest as the subplot, and treatments as the sub, subplot.

Results and Discussion

Cellulase had a greater effect on cell-wall degradation in orchardgrass (30%) than in alfalfa silage (14%) due to greater lignification of alfalfa at the same maturity. Concentration of NDF decreased significantly with increased cellulase concentration from 0 to 20 mL/kg in orchardgrass and from 0 to 10 mL/kg in alfalfa (Fig. 1). In both species, cellulose concentration was reduced more than that of hemicellulose. Because of increased lignification at late maturities, cellulase generally was less effective on more mature forages (Fig. 1 and 2). Pectinase, inoculant, and formic acid did not affect cell-wall concentration of the silages (data not shown). Although there was up to 30% reduction in NDF concentration by cellulase, there were no consistent differences in IVDMD among treatments. However, formic acid tended to increase IVDMD of cellulase-treated grass silage. Additionally, ensiling reduced IVDMD concentration in both species (data not shown).

Conclusion

Addition of cellulase reduced cell-wall concentration of the silage, which could improve DM intake and digestibility. A feeding trial on sheep is currently being conducted to study these effects.

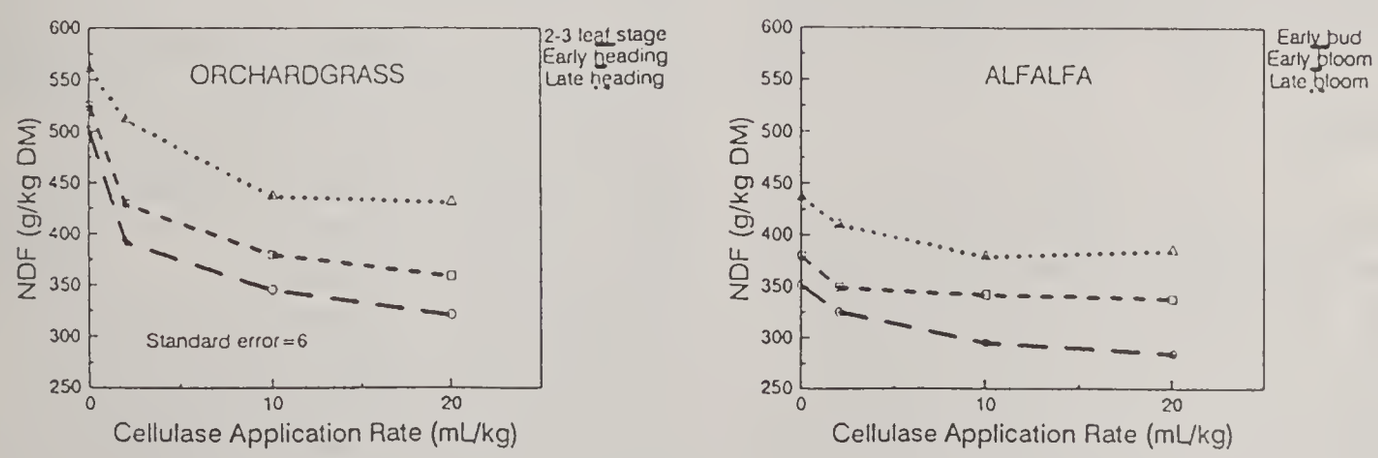


Figure 1. Effect of cellulase application rate on neutral detergent fiber (NDF) concentration of orchardgrass and alfalfa silages harvested at three maturities. Data are average of four field replicates and two growth cycles.

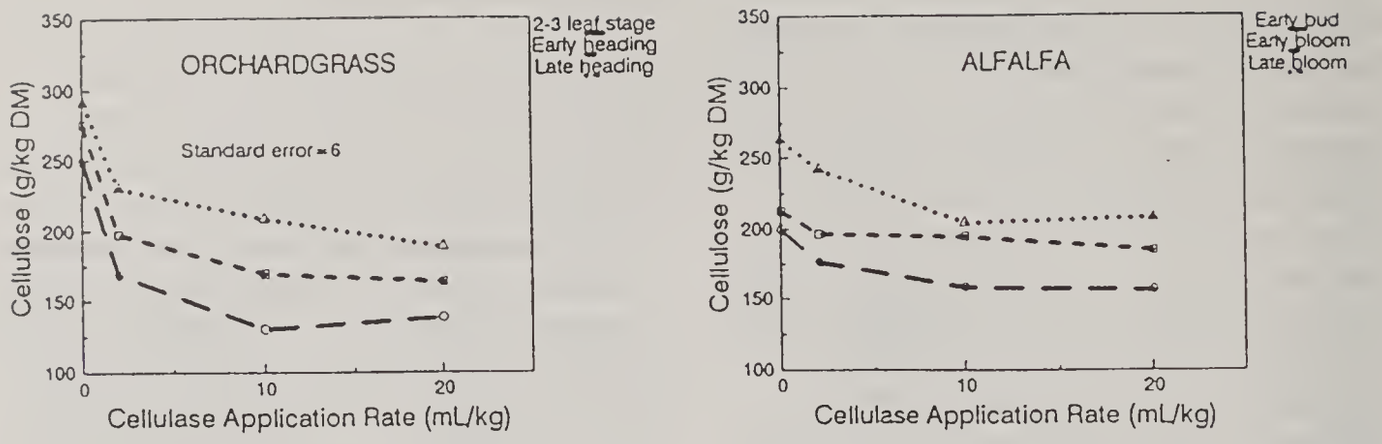


Figure 2. Effect of cellulase application rate on cellulose concentration of orchardgrass and alfalfa silages harvested at three maturities. Data are average of four field replicates and two growth cycles.

Ensiling of Frozen High Moisture Shelled Corn

R.E. Muck and T. Kriegl

Introduction

Weather conditions in 1992 were such that farmers had difficulty ensiling high moisture corn at normal times. Many farmers were faced with ensiling corn at too high a moisture content in the fall or leaving the crop in the field and harvesting in the winter. The objective of this study was to monitor the quality of high moisture shelled corn that had been harvested and ensiled frozen in January or February.

Methods

Four silos on four different private farms in Sauk County, WI were selected for monitoring. Three silos were steel oxygen-limited silos; the fourth was a concrete stave silo. In each case, the corn had been harvested in January or February. A bacterial inoculant was added to corn entering one of the oxygen-limited silos. No additives were applied at ensiling in the other three.

From April 2 until July 16, farmers collected a sample of the high moisture corn once every three weeks during normal daily unloading of their silos. Samples were immediately frozen and kept so until analyzed. Samples were analyzed for moisture content, pH, lactic acid bacteria, yeasts, molds, fermentation products and aerobic stability.

Results and Discussion

Comparisons of the high moisture shelled corn from the first and last samplings at the four farms are shown in Table 1. The three oxygen-limited silos (Farms R, T, W) had corn that was virtually unfermented when sampling started whereas fermentation had already occurred in the concrete stave silo. Intermediate points for the oxygen-limited silos indicated a gradual increase in the number of lactic acid bacteria with a subsequent increase in fermentation products and decrease in pH. By the last sampling of the oxygen-limited silos, fermentation appeared to be nearly complete

as there had been little change in pH and fermentation products from the previous sampling. In the concrete stave silo, decreases in pH and increases in lactic acid were observed, but these appeared to be more related to the increased moisture content of the corn removed in June and July compared with that removed earlier.

Yeasts and molds in the oxygen-limited silos generally increased with time. As these populations increased, aerobic stability or bunk life decreased. In the concrete stave silo, yeast and mold counts started high and remained so throughout sampling. By summer, bunk life was short for the high moisture corn coming from all silos although the driest corn was consistently the most stable.

Farm T had inoculated its high moisture corn at ensiling. In absence of a control for comparison, one cannot make any substantive conclusions. However, the inoculated corn certainly was not superior to the others. By comparison with Farm R that had a similar silo and corn at a similar moisture content, there was little difference in fermentation rate or pattern, and bunk life was somewhat better in the uninoculated silo in April and May.

Conclusions

From these results, it appears that high moisture corn can be successfully ensiled even if it is harvested frozen. Once the corn begins to thaw in the spring, lactic acid bacterial counts rise, and fermentation begins and proceeds normally. The one caveat of ensiling frozen high moisture shelled corn seems to be aerobic stability or bunk life. By summer, bunk life was very short in corn from all four silos. Monitoring of silos in the future is necessary to confirm that this is indeed a problem.

Table 1. Characteristics of the high moisture corn at four farms for the first and last sampling dates.

	Farm R		Farm T		Farm W		Farm Z	
	4/2	7/16	4/2	7/16	4/2	7/16	4/16	7/16
Moisture, %	27.3	27.3	29.7	26.3	20.1	20.5	28.5	34.3
pH	6.27	5.22	6.44	5.52	6.54	5.95	5.34	4.76
Lactic Acid[1]	0.04	0.34	0.00	0.27	0.00	0.06	0.31	1.29
Acetic Acid[1]	0.06	0.21	0.06	0.16	0.08	0.05	0.18	0.08
Propionic Acid[1]	0.00	0.12	0.00	0.20	0.00	0.02	0.16	0.00
Ethanol[1]	0.00	0.31	0.00	0.10	0.00	0.03	0.00	0.00
Lactic Acid Bacteria[2]	6.74	8.38	5.99	8.05	<2.00	7.41	7.29	7.24
Yeasts[2]	4.65	5.94	4.80	6.79	3.83	6.68	7.11	7.35
Molds[2]	3.02	4.64	4.54	6.49	4.40	5.30	6.85	5.77
Aerobic Stability, h	15.	5.	4.	5.	121.	12.	4.	2.

[1]Fermentation products in % DM.
[2]Microbial counts in log (colony forming units/g corn).

The Importance of Cell Wall Cross-Linking

J. Ralph, S. Quideau, J.H. Grabber, R.D. Hatfield and H.G Jung

Introduction

Effective separation and/or utilization of plant cell wall polysaccharides is often thwarted by their intimate association with the phenolic polymer, lignin. The difunctional 4-hydroxycinnamic acids, notably *p*-coumaric **1** and ferulic **2** acids (Fig. 1), in forage legumes and grasses are capable of cross-linking cell wall components and logically have a role in

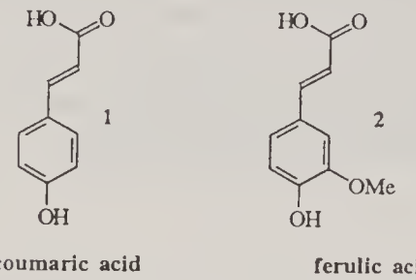


Figure. 1. p-Coumaric and ferulic acids, hydroxycinnamic acids which are precursors of the lignin monomers are important in cell wall cross-linking.

inhibiting the digestion, by ruminants, of potentially degradable polysaccharides. For example, 170 h *in vitro* digestion of forage materials results in indigestible residues which typically contain 60-70% polysaccharides. Analysis shows that the carbohydrate monomer composition is surprisingly similar to that of the original feed. In the forage cell wall, covalent bonds that cannot be enzymatically hydrolyzed create regions of restricted access where degradative enzymes cannot penetrate. Researchers have postulated the effects of cross-linking on plant cell wall properties such as accessibility, extensibility, plasticity and digestibility. It is to understand the structural factors limiting digestibility that many researchers are pursuing studies involving the cross-linking of polysaccharides and lignin by hydroxycinnamic acids.

Ferulic acid in grasses is implicated in cross-linking cell wall carbohydrates to lignins. Its attachment to carbohydrates, as esters such as **3** (Figs. 2, 3) is relatively well defined, and an understanding of its attachment to lignin is developing. The determination of the nature and scope of lignin-hydroxycinnamic acid-polysaccharide interactions in plant cell walls is critical towards our understanding of cell wall biogenesis and degradation. For example (Fig. 1), ferulate esters **3** can become 'passively' involved in cross-linking by trapping lignin quinone methide intermediates **4** and/or can be 'actively' involved (Fig. 3) in the free-radical polymerization process. In the former process, simple α-etherified structures **5** result, whereas co-polymerization with lignin monomers results in a variety of structures including **9-14**

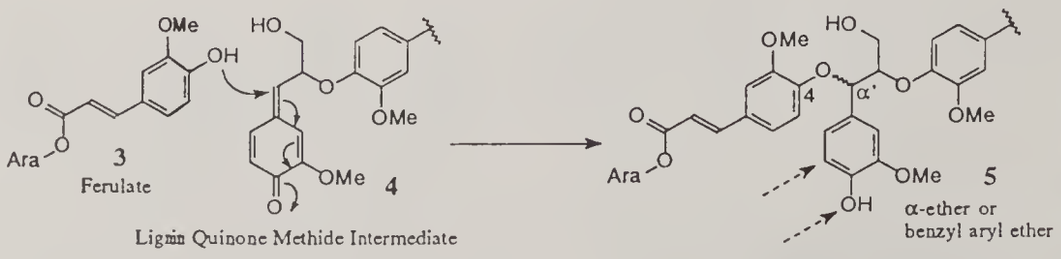


Figure 2. 'Passive' coupling of polysaccharides to lignin via polysaccharide ferulate ester attack on lignin quinone methide intermediates. Note (from Fig. 3) that production of the quinone methide 4 requires oxidative phenolic coupling mechanisms but that ferulate addition is a purely chemical nucleophilic addition — by this mechanism, ferulate does not enter oxidative coupling reactions.

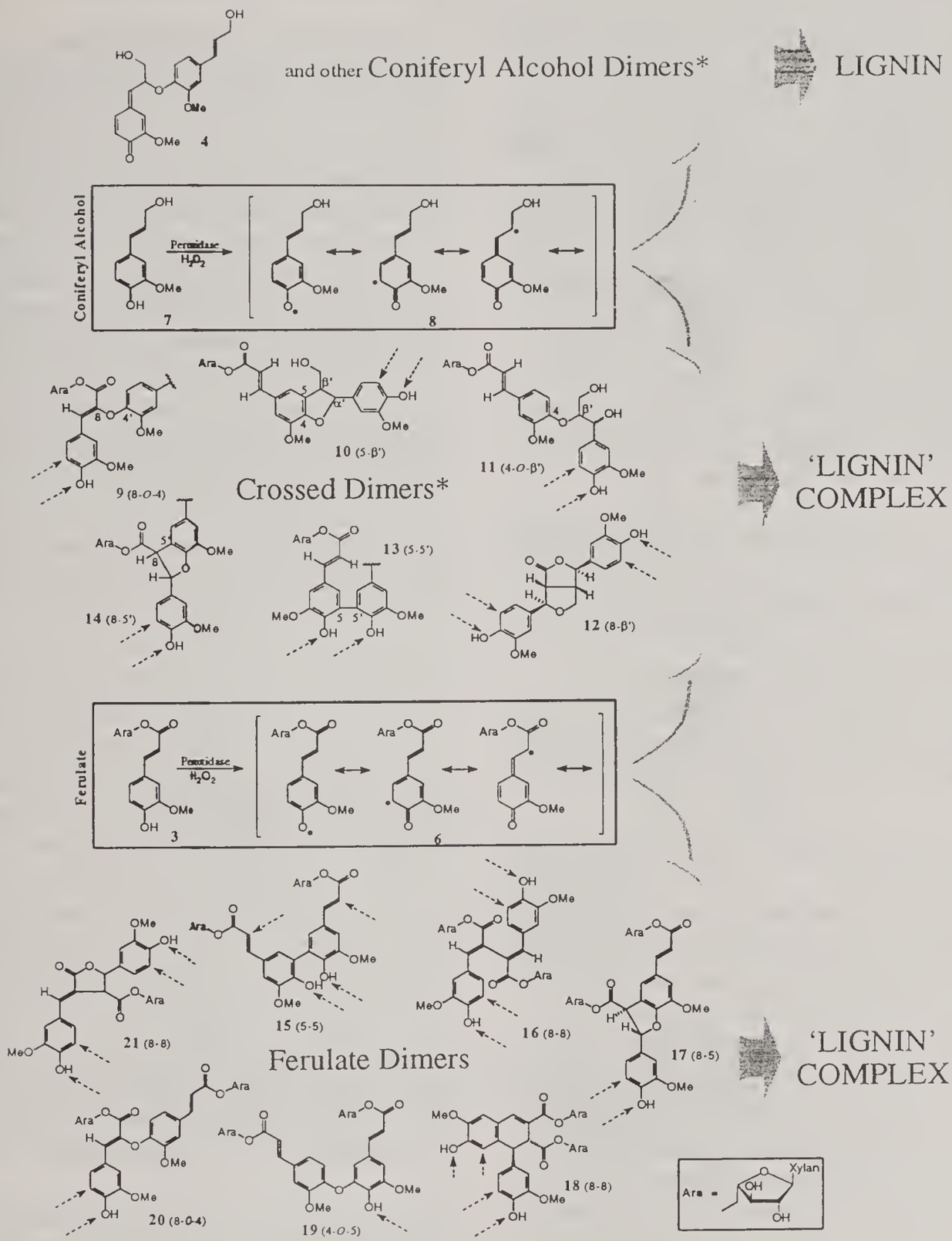


*Figure 3. Phenolic oxidation pathways for ferulate monomers (shown here as ferulate arabinoxylan esters) in the presence of lignin monomers (coniferyl alcohol in this case). Coniferyl alcohol **7**, by itself, dimerizes (including to the β-O-4) quinone methide dimer **4**) and polymerizes to lignin. Ferulate can oxidatively dimerize to give a range of dehydrodimers **15-21** (bottom section) and these can couple with coniferyl alcohol radicals (at arrowed sites) to incorporate into the lignin complex. Ferulate can couple with lignin monomer or oligomer radicals (central section) to give a variety of crossed dimers (or oligomers) including **9-14** which also incorporate into a lignin complex by further coupling with coniferyl alcohol monomer or oligomer radicals. Dotted arrows indicate sites for further radical coupling. * Indicates that higher oligomers as well as dimers are applicable here.*

(Fig. 3), only some of which would be subsequently identifiable, by present solvolytic methods, as arising from ferulic acid. What is often overlooked is that the frequently cited 'passive' mechanism, Fig. 2, requires the presence of enzymes and substrates for oxidative coupling of lignin monomers to form the requisite quinone methide **4**, but deny the ferulate **3**, also a conjugated phenol, the option of such oxidative coupling — it must simply sit around until it can react with one of these quinone methides. By contrast, 'active' incorporation (Fig. 3), over which the plant has much greater control, leads to very efficient cross-linking (with the notable exception that 8-β coupling results in displacement of the polysaccharide chain – structure **12**).

Under-appreciation of the extent of cross-linking.

For a number of reasons, the extent and importance of cross-linking by hydroxycinnamic acids has not been fully appreciated despite some outstanding research in this area. This is in part attributable to the following three factors.

1. Under-appreciation of 'active' incorporation mechanisms.

Although the idea of active incorporation (Fig. 3) of ferulate esters **3** into the lignin matrix by oxidative coupling of phenols has appeared in the literature, this mechanism is essentially ignored to this day in favor of 'passive' incorporation (Fig. 2). We have proven that appropriate ferulate esters will actively incorporate efficiently into a synthetic lignin polymer producing a complex but predictable array of structures **9-14** (and others) and have gone so far as to state recently that "it is difficult to imagine how 4-hydroxycinnamoyl monomers and dimers can be present in the cell wall for addition to quinone methides and yet not be amenable to radical coupling processes." What is clear is that ferulic acid will not be released from many of these structures by normal solvolytic analysis methods and that the potential for underestimation of ferulic acid in lignified plant materials is substantial.

2. The importance of ferulate ester dehydrodimers has been underestimated.

It has been considered that dehydrodimers represent only a small fraction of the ferulic acid in the cell wall. We have recently shown that this is far from the case in reality (see the accompanying article on the identification of new ferulic acid dehydrodimers). Again, despite excellent work in this area, two factors have led to an enormous underestimation of these dimers. (i) The GC or whole-procedure response factor for released 5-5-coupled dehydrodiferulic acid (from dimer **15**), universally ignored or assumed as unity, is significantly lower (0.22) than for the monomers or internal standards used (1.00). Consequently, the measured amounts need a response-factor correction which would yield higher values by a factor of ca. 5. (ii) Only one ferulic acid dehydrodimer, the 5-5-isomer (from **15**) has previously been reported or quantitated. In reality, there are 7 isomeric dehydrodiferulic acid dimers which can be released by base solvolysis from dehydrodimers **15-21** formed by oxidative coupling of ferulate esters. We have recently shown that all are indeed found in plant tissues and that the amounts of most of these substantially exceed the amount of the 5-5-coupled dimer (see accompanying article). In fact, the sum of all the dehydrodimers can be over 20 times the amount of the 5-5-dimer alone. Obviously, these dehydrodimers have been seriously underestimated by analytical methods which determine only the 5-5-isomer.

By dealing with these two factors, our studies have shown that up to 50% of the total ferulic acid in the cell wall may be in the form of dehydrodimers and that the dimers can be formed before the onset of lignification. Additionally, in mature plant tissues, the dehydrodiferulic acids are incorporated into the lignin structure and, as mentioned for ferulic acid above, this cannot be completely

released for quantitation; only α- and β-ethers are releasable.

3. There is little recognition of the unique role dehydrodimers can have in cross-linking the cell wall.

Although the hydroxycinnamate monomers **3** are capable of cross-linking a polysaccharide to lignin, Fig. 4, dehydrodimers **15-21** (and, in fact the photochemically derived cyclodimers) are capable of simultaneously cross-linking two polysaccharide chains and the lignin polymer, Fig. 5. Cross-linking of the lignin is additionally possible for some of the dehydrodimers. This type of extensive cross-linking is likely to have a major impact on the properties of the cell wall, but the implications and structural chemistry have not been addressed.

Conclusion

The importance and the impact of cell wall cross-linking have long been considered important but only recently has it become apparent that its importance has been severely underestimated. Current efforts involved in ascertaining the detailed chemistry and biochemistry of cross-linking should allow rational approaches to plant modification for improved digestibility.

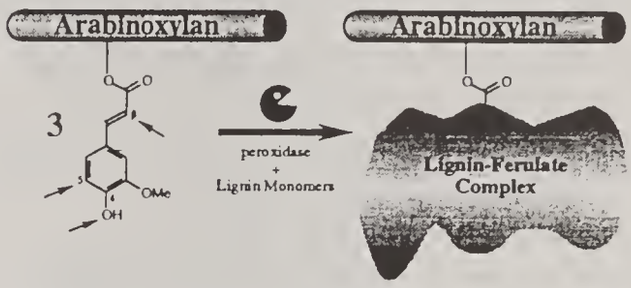


Figure 4. Coupling of polysaccharide ferulate esters via active mechanisms incorporate the ferulate moiety into the lignin complex via bonding to the β-, 5-, and 4-positions. Only some of the ferulate is releasable by ether-cleaving reactions.

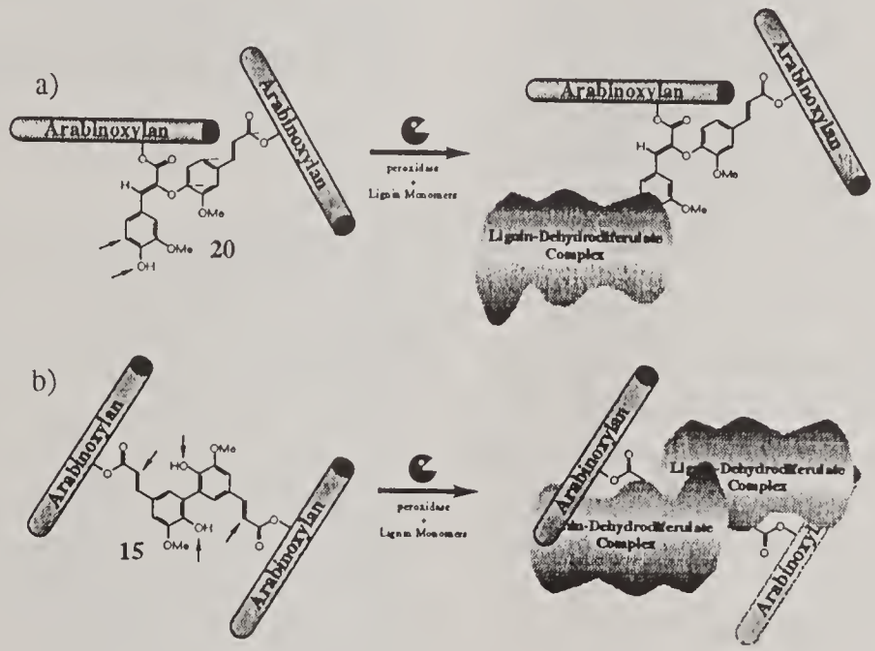


*Figure 5. Ferulate dehydrodimer formation has already effected the cross-linking of polysaccharide chains. Further cross-linking with lignin can occur via 'passive' or 'active' incorporation into the lignin polymer complex. Via active mechanisms (oxidative coupling of phenols), 8-O-4 aryl ether dimer **20** (Fig. a) and, in fact dimers **16-21**, incorporate to give highly cross-linked cell wall structures. Dimer **15**, the 5-5-coupled dimer, having phenolic and 8-positions accessible for coupling, can form an enormous variety of highly cross-linked structures, even cross-linking the lignin polymer (or forming branch-points). In 8-position coupling, however, some products can lose the polysaccharide chain by internal transesterification by the same process as in the formation of crossed ferulate-coniferyl alcohol dimer **12** (Fig. 3). The relative importance of these dimers in coupling and in retaining their polysaccharide connections requires investigation.*

Identification of New Ferulic Acid Dehydrodimers in Grass Cell Walls

J. Ralph, S. Quideau, J.H. Grabber and R.D. Hatfield

Introduction

Ferulic acid is esterified to graminaceous cell wall polysaccharides, notably to arabinoxylans at the primary position of α-L-arabinofuranoside moieties. Dimerization of such feruloyl esters can provide a pathway for cross-linking polysaccharide chains. Surprisingly, the only *established* dehydrodimer, released in small amounts from grass cell walls by saponification, is the 5-5-coupled dehydrodiferulic acid **16** (referred to as "diferulic acid"). Although it has been unambiguously established that such structures do indeed cross-link polysaccharides, we have been unsuccessful, using either enzymic or metallic one-electron oxidations, in eliciting substantial proportions of 5-5-coupled dehydrodimers from feruloyl esters. This was entirely expected from the observation that radical coupling products involving the 8-position and, in particular, 8-5 coupling products invariably predominate from oxidative dimerization of (*E*)-4-(prop-1-enyl)phenol derivatives by a variety of methods.

It was these basic studies on feruloyl ester oxidative dimerization that led us to speculate that, if dehydrodiferuloyl esters were produced *via* radical coupling mechanisms, other feruloyl ester dehydrodimers (structures **8**, **9**, **11** and **12** in Fig. 1) should be present in the cell wall, and potentially in greater amounts than the observed 5-5-coupled dehydrodiferuloyl moiety **10**.

Methods

Saponification of cell walls. Cell walls (50-100 mg) were treated for 20 h at 25°C with 2 N NaOH under nitrogen. 2-Hydroxycinnamic acid (0.1 mg) was added as an internal standard. Samples were acidified with 12 N HCl and extracted into ether. Dried extracts were silylated with pyridine and N,O-bis(trimethylsilyl)trifluoroacetamide. Trimethylsilylated (TMS) derivatives of phenolic acids were separated by GC. GC response factors were determined for *p*-coumaric, ferulic, and each of the dehydrodiferulic acids following silylation. Response factors through the entire saponification procedure, using standards in the presence of cellulose, were comparable to directly determined GC response factors but were more variable. Recovery was often extremely poor without added cellulose. For mass spectrometry, silylated samples were separated with GC and detected with an HP 5970 mass selective detector.

Synthesis of all dehydrodiferulic acid isomers required for authentication.
This is described elsewhere in this summary.

Discussion

Compounds 13-16 and **18-19**, the 8-5, 8-8, and the 8-*O*-4 dimers (Fig. 1), were readily apparent in extracts from a number of plant materials (Figs. 2 and 3). The 4-*O*-5 dimer **17** may also be present in some samples at very low concentration — the retention time of a small peak occasionally observed in this region matches exactly, but authentication from mass spectrometry was not possible due to its low abundance. What is particularly striking is that the amounts of dimers **13+14** (8-5), **15** (8-*O*-4), and **18+19** (8-8) match or exceed those of the only previously reported dimer, the 5-5 dimer **16** (Fig. 3).

It is surprising that these compounds have not been reported or identified previously although the percentage of each can vary substantially. From the plant materials surveyed (Fig. 3), the

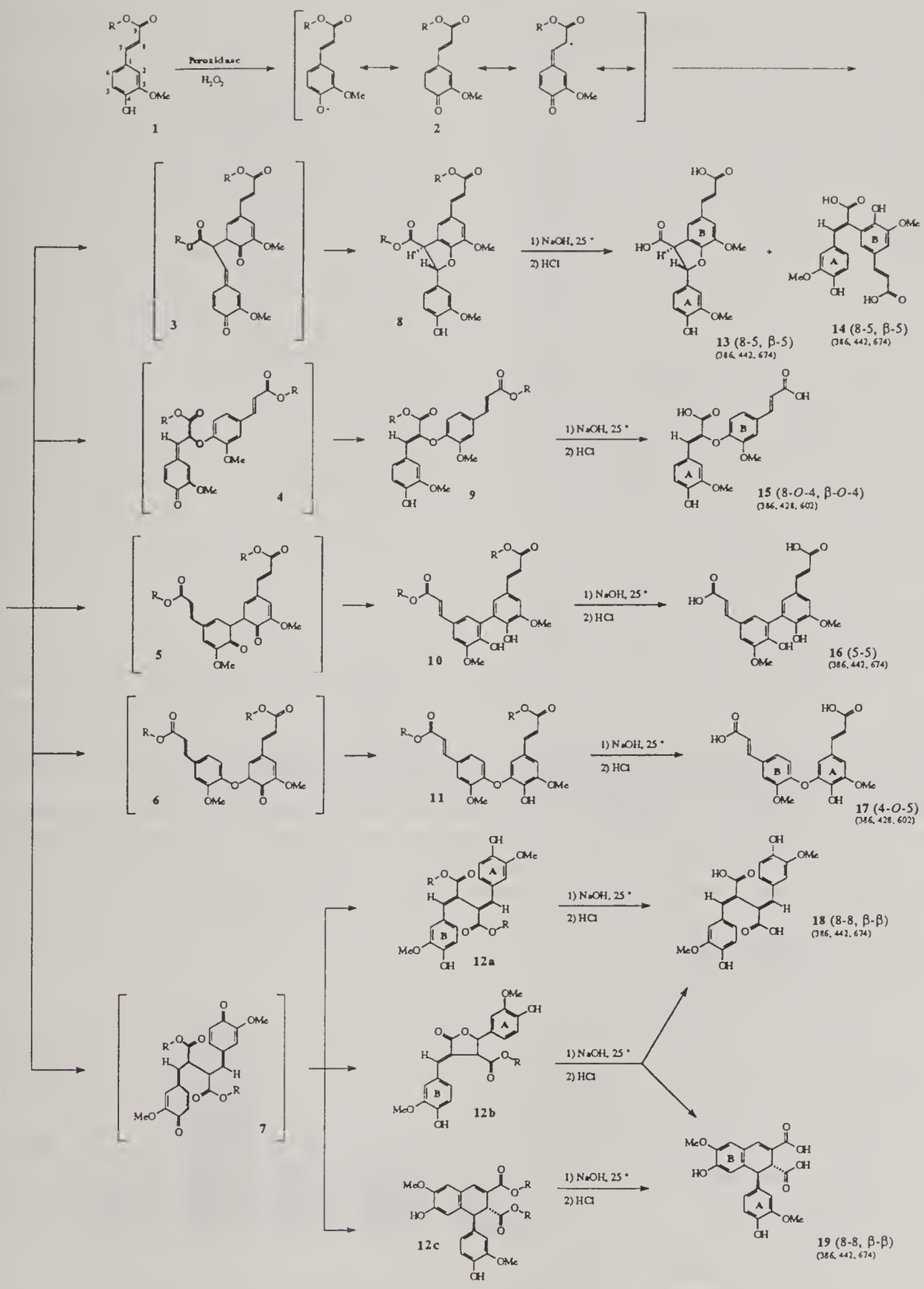


Figure 1. The general chemistry of dehydrodimer formation and saponification. Dimerization of feruloyl esters via phenoxy radical **2** *gives intermediates* **3-7** *which react in the cell wall to form dehydrodiferuloyl esters* **8-12**. *During chemical analysis* **8-12** *can be saponified to dehydrodiferulic acids* **13-19**. *The values in parentheses below the structures represent the nominal masses for the parent compound, the fully methylated, and the fully trimethylsilylated derivatives, respectively.*

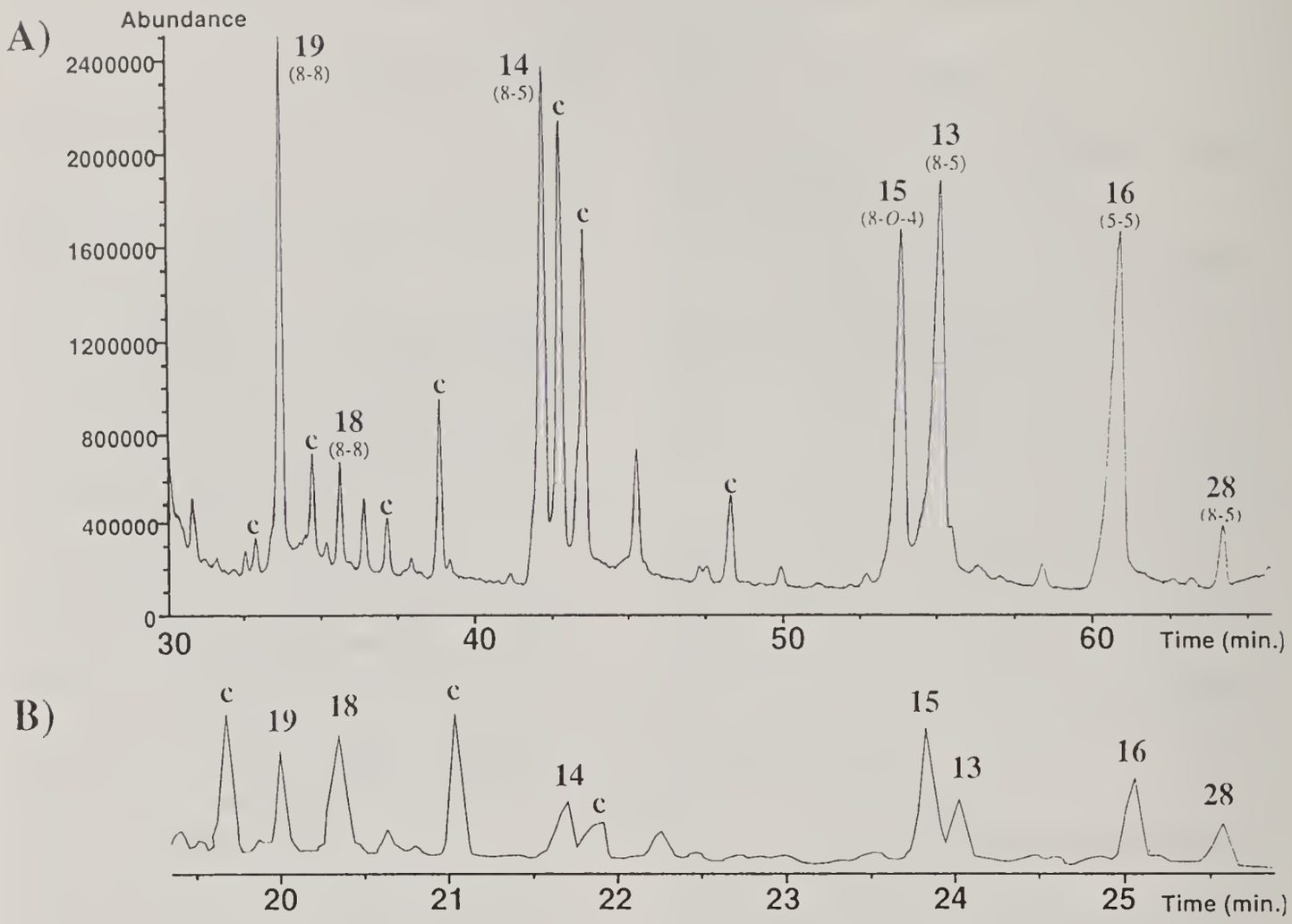


*Figure 2. GCs of dimer regions of saponified cell wall extracts. A) Total ion chromatogram from GC-MS of the dimers region of a saponified extract of primary cell walls from suspension cultured corn showing dehydrodiferulic acids **13-19** (with exception of **17**). Peaks labeled **c** are assigned, without further authentication, to cyclodimers by observation of an m/z 338 peak in their mass spectra. B) Trace from a shorter GC/FID run of switchgrass parenchyma dehydrodimers — the relative retention times vary slightly compared to the trace in A due to the temperature program used. Amounts determined from GC are given in Table 1.*

total amount of FA dimers was seven- to twenty-fold higher than the amount of the 5-5 dimer alone. It is also apparent that ferulic acid dehydrodimers can constitute a substantial fraction of the ferulic acid in the cell wall. Any comparisons of the relative contributions of monomers, cyclodimers and dehydrodimers in the future must include the suite of dehydrodimers reported here and should address the disparate response factors for these compounds.

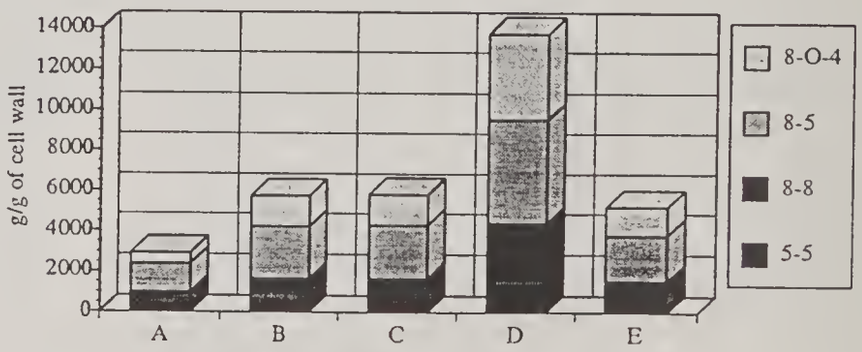


Figure 3. Composition (mg/g of cell wall material) of dehydrodimers deriving from the individual coupling modes (5-5, 8-8, 8-5, and 8-O-4) for various plant cell wall samples: A) suspension-cultured corn, B) orchardgrass parenchyma, C) orchardgrass sclerenchyma, D) switchgrass parenchyma, E) switchgrass sclerenchyma.

Synthesis of Dehydrodiferulic Acids

S. Quideau, J. Ralph and J.H. Grabber

Introduction

Until quite recently, the only established dehydrodiferulic acid released in small amounts from grass cell walls by saponification was the 5-5-coupled dehydrodiferulic acid. Our initial thought on feruloyl ester oxidative dimerization led us to speculate that, if dehydrodiferuloyl esters were produced via oxidative coupling mechanisms, other feruloyl ester dehydrodimers should be present, and potentially in greater amounts than the 5-5-coupled dehydrodiferuloyl ester. Indeed, GC/MS analysis of cell wall materials indicated the presence of several possible ferulic acid-derived dimers. The synthesis of all anticipated ferulic acid dehydrodimers was thus required for authentication purposes.

Synthesis of all dehydrodiferulic acid isomers required for authentication (c.f. structures **13-15** and **17-19** in "Identification of New Ferulic Acid dehydrodimers in Grass Cell Walls" by Ralph *et al.* in these Research Summaries).

Method Highlights and Discussion

The diester phenylcoumaran (R = Et) was synthesized in about 30% isolated yield from ethyl (*E*)-ferulate using Ag_2O oxidation, Fig. 1. Treatment of this diester with 2M sodium hydroxide at room temperature for ca. 20 h produced the diacid stilbene (*E,E*)-**14** and the diacid phenylcoumaran (*E*)-**13**. Alternatively, **14** was more efficiently prepared by the action of the strong non-nucleophilic base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) on the diester phenylcoumaran (R = Et), followed by saponification, Fig. 1.

The key step in the synthesis of the 8-*O*-4-coupled dehydrodiferulic acid **15**, Fig. 2, is the generation of the aldehydic β-hydroxy ester intermediate via aldol reaction. Nucleophilic displacement by trimethylsilyl bromide afforded the corresponding benzyl bromide. Treatment with DBU gave the single isomer of the aldehydic styryl ether shown in Fig. 2 via in situ generation of a quinone methide intermediate and subsequent 8-proton elimination. Subsequent conversion of the aldehyde to a methyl ester group was then cleanly accomplished by use of the Corey-Gilman-Ganem oxidation procedure. Saponification finally gave the required (*Z,E*)-8-*O*-4-coupled dehydrodiferulic acid **15**.

Literature routes to 4-*O*-5 compounds are lengthy. We report here an extremely convenient route, Figure 3, to the 4-*O*-5-coupled

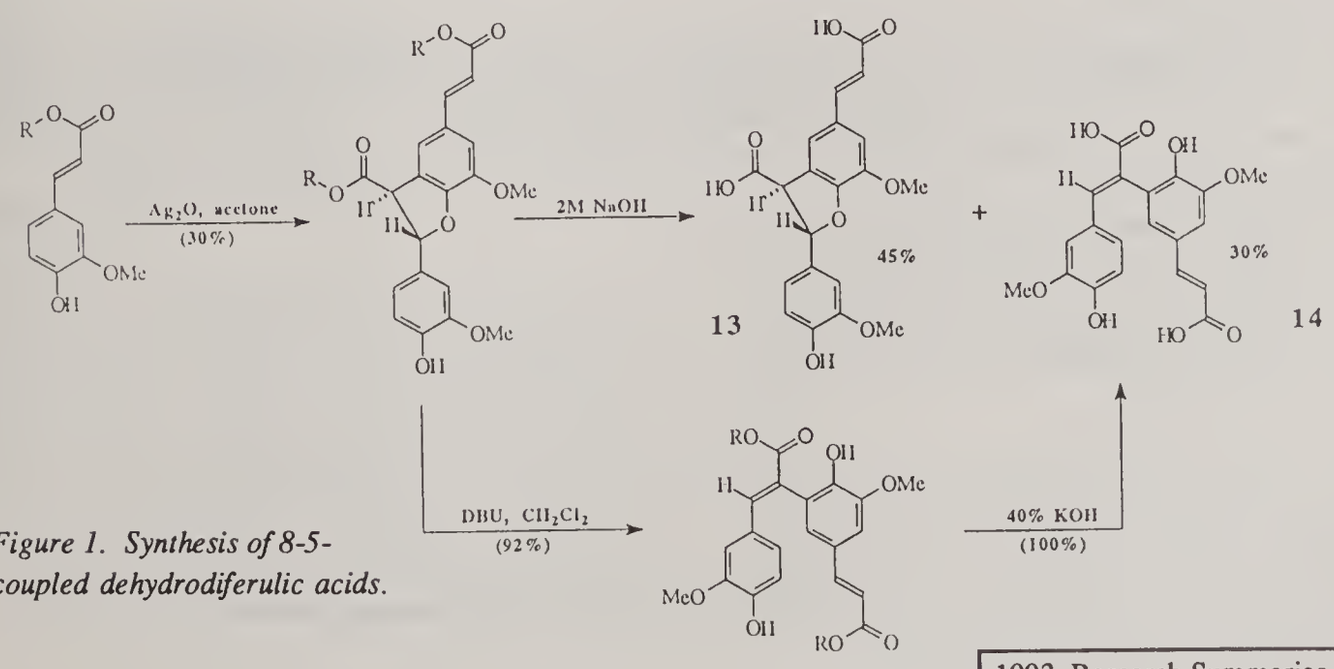


Figure 1. Synthesis of 8-5-coupled dehydrodiferulic acids.

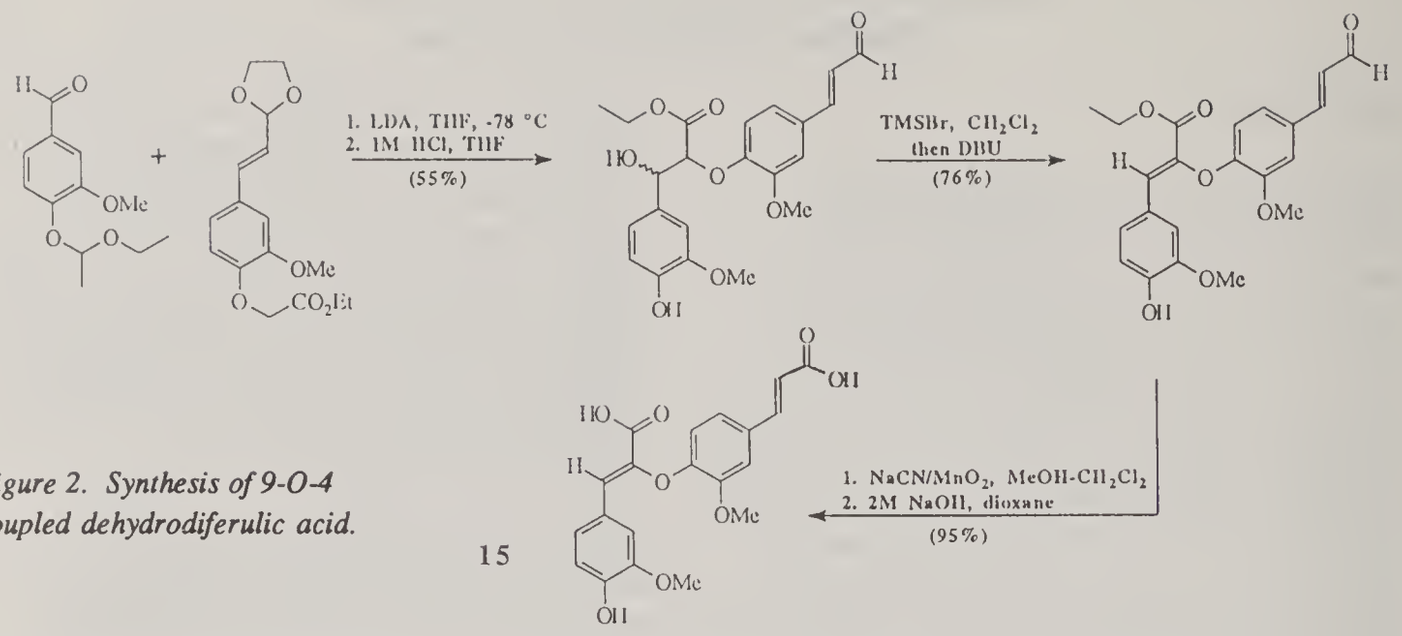
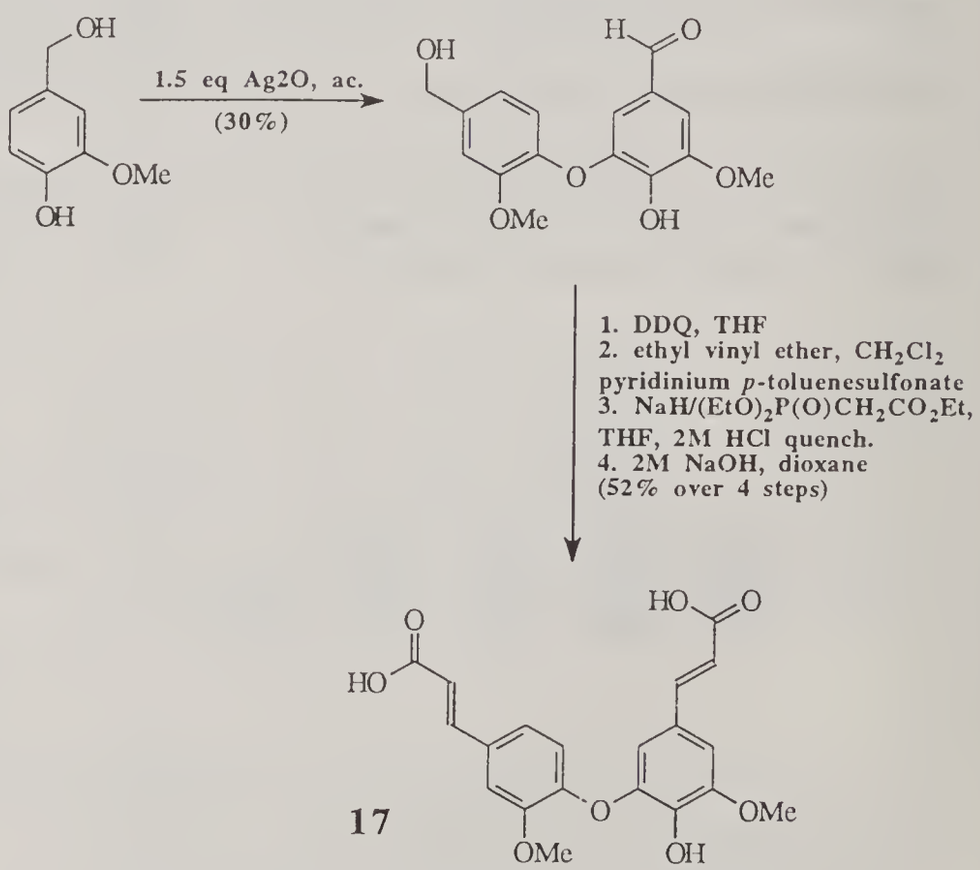


Figure 2. Synthesis of 9-O-4 coupled dehydrodiferulic acid.

dehydrodiferulic acid **17**, based again on Ag_2O-mediated oxidation. We felt that if a 4-*O*-5-coupled dehydrodivanillin was obtained by a simple oxidative radical coupling process, subsequent addition of the remainder of the side-chain would give us the desired 4-*O*-5-coupled dehydrodiferulic acid **17**. Although selective coupling at the 5-position is observed upon enzyme-catalyzed oxidation of vanillin, Ag_2O-mediated oxidation rapidly led to a complex mixture of oligo/polymeric materials. We thus suspected that vanillyl alcohol, as a less conjugated structure than vanillin, could be more appropriate to undergo 4-*O*-5 coupling. Indeed, reaction of vanillyl alcohol with 1.5 eq of Ag_2O in acetone produced the mixed 4-*O*-5-coupled product shown in Fig. 3 as the main dehydrodimer in up to 30% yield. A conceivable selective coupling between vanillyl alcohol at the 4-position and vanillin at the 5-position could explain the formation of such a mixed coupled product. Oxidation with DDQ gave a 4-*O*-5-coupled dehydrodivanillin. Protection and completion of the two side-chains by a Wittig-Horner reaction using triethylphosphono-acetate, and subsequent deprotection and saponification gave the (*E,E*)-4-*O*-5-coupled dehydrodiferulic acid **17**, Fig. 3.



17

Figure 3. Synthesis of 4-O-5-coupled dehydrodiferulic acid.

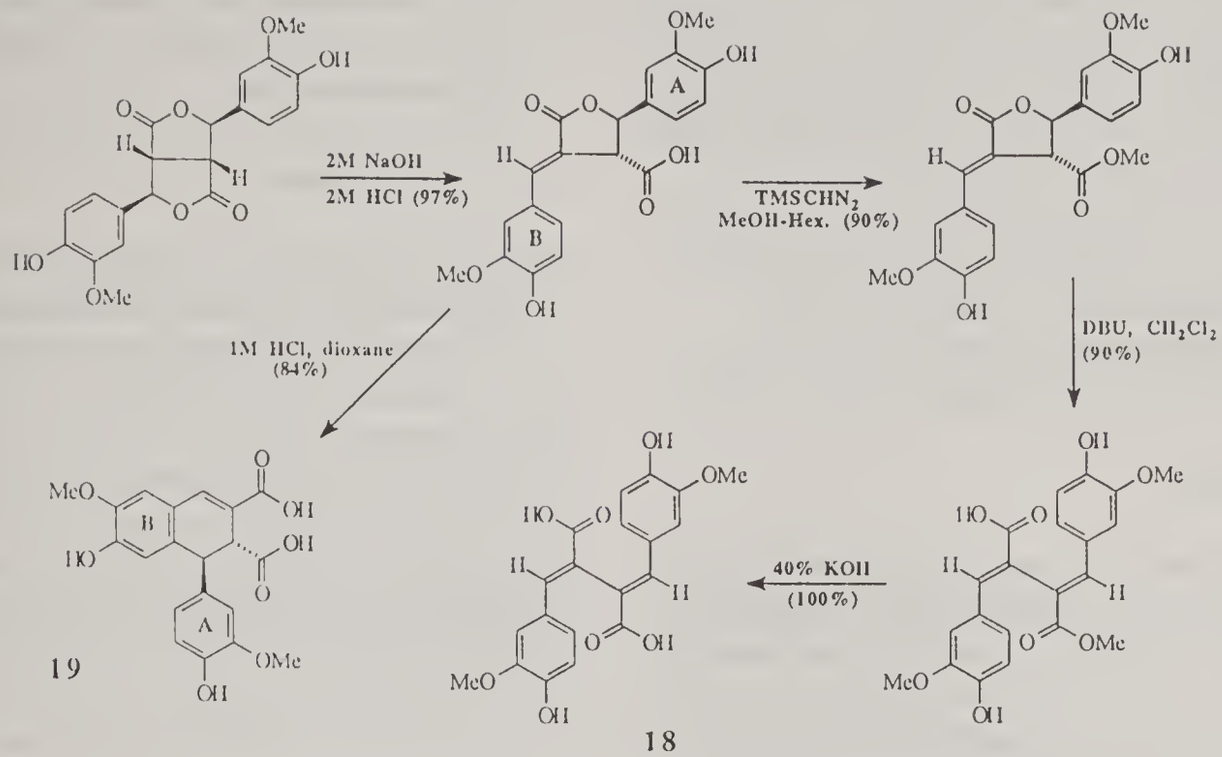


Figure 4. Synthesis of 8-8-coupled dehydrodiferulic acids.

The lignans **18** and **19** were both derived from the dehydrodiferulic acid dilactone 4-*cis*,8-*cis*-bis(4-hydroxy-3-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octan-2,6-dione, Fig. 4. Alkali treatment gave rise to a carboxylic acid γ-monolactone upon acid work-up. Selective methylation of the carboxylic acid function was accomplished by using trimethylsilyldiazomethane. The esterified γ-lactone was then well-suited for generation of a bis-α,β-enone system via DBU-mediated elimination affording a bis-benzylidene succinic acid monomethyl ester derivative, whose saponification gave the 8-8-coupled dehydrodiferulic acid **18** (presumably the (*E,E*)-isomer). The initial carboxylic acid γ-monolactone was found to cleanly rearrange to the desired 1-aryl-*trans*-1,2-dihydronaphthalene derivative **19** upon treatment with HCl in refluxing dioxane.

Model Studies of Lignin-feruloyl Ester Cross-linking and Fiber Degradation of Corn

J.H. Grabber, A. Pell, S. Quideau, J. Ralph, R.D. Hatfield and N. Amrhein

Introduction

Feruloyl esters are thought to play a major role in limiting fiber degradation, due to their demonstrated ability to cross-link structural polysaccharides and lignin. Ferulic acid is esterified to arabinoxylans by Fer-CoA transferase during cell-wall synthesis. Selecting or engineering plants with low Fer-CoA transferase activity would reduce feruloyl ester deposition and incorporation into lignin. Before initiating such work, we must determine if fiber degradation is improved by reduced cross-linking of feruloyl esters to lignin.

This summary describes the use of suspension cultured corn to model lignin-feruloyl ester cross-linking in grasses. Isolated cell walls were synthetically lignified to form cell-wall

dehydrogenation polymers (CWDHP). Feruloyl ester concentrations in cell walls were reduced by adding 2-aminoindan-2-phosphonic acid (AIP) to the culture medium. We made CWDHP from normal and AIP treated cultures to 1) evaluate how well synthetic cell-wall lignins model grass lignins, 2) determine how extensively feruloyl esters are incorporated into lignin, and 3) determine if fiber degradability is improved by a moderate reduction in lignin-feruloyl ester cross-links.

Methods

Suspension cultures of Black Mexican sweet corn were grown with 0 and 10 μM AIP, to produce walls with normal and low concentrations of feruloyl esters, respectively. Isolated cell walls were suspended in PIPES buffer (50 mM, pH 6.5) and synthetically lignified by the polymerization of coniferyl and sinapyl alcohols by cell-wall peroxidase (see the 1992 Research Summary). Unlignified controls were incubated in buffer only. Treatments were replicated three times.

Structural characteristics of CWDHP lignins were evaluated by thioacidolysis and ^{13}C-NMR. Syringyl and guaiacyl monomers and dimers released by thioacidolysis were silyated and quantified by GC. A modified 'milled wood lignin' (MWL) was isolated by extracting ball milled and cellulase treated CWDHP with dioxane/water. Proton-decoupled ^{13}C-NMR was run under standard conditions using 30 mg of EDTA-washed CWDHP lignin in 9:1 acetone-d_6:D_2O. Lignin content of cell walls was estimated by a modified Klason procedure. Samples were treated sequentially with 2 N NaOH (20 h at room temperature) and 4 N NaOH (2 h at 170°C) to release ester- and ether-linked hydroxycinnamic acids. Hydroxycinnamic acids extracted after each step were silylated and analyzed by GC. Gas production curves from in-vitro fermentations were fitted with a modified Gompertz equation to estimate cell-wall digestion kinetics.

Results and Discussion

Unlignified cell walls contained very low concentrations of esterified *p*-coumaric acid (~ 0.2 mg g^{-1}) and Klason lignin (<10 mg g^{-1}). Synthetic lignification increased Klason lignin concentrations to ~ 110 mg g^{-1}. Synthetic cell-wall lignins were structurally similar to grass lignins according to thioacidolysis (Table 1). Lignin isolated from CWDHP had broad ^{13}C NMR spectra characteristic of grass lignins (Fig. 1). These results suggest that synthetic cell-wall lignins are a reasonable model of natural grass lignins.

The quantity of total feruloyl esters (monomers plus dehydrodimers) in unlignified walls were reduced from 18.0 mg g^{-1} in normal cultures to 8.1 mg g^{-1} in AIP treated cultures. Dehydrodiferulic acids comprised about 50% of total feruloyl esters which is remarkable considering the low concentration of ferulic acid in cell walls. Dehydrodimers in unlignified cell walls were coupled by β-5 (50%), b-O-4 (20%), 5-5 (15%) and β-β (15%) linkages. Lignification reduced the concentration of total feruloyl esters released

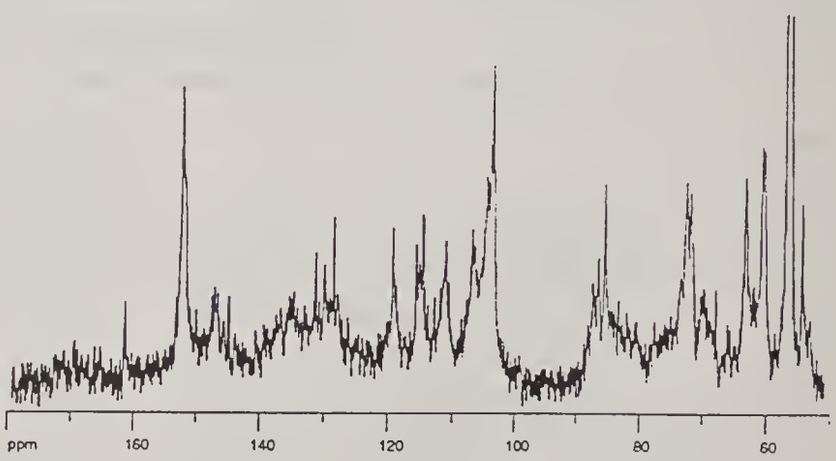


Figure 1. ^{13}C-NMR spectrum of synthetic cell-wall lignin.

by saponification by 85 to 95% (Table 2). About 50% of total feruloyl esters incorporated into lignin were cross-linked by ether bonds. This suggests that current solvolytic methods underestimate lignin-feruloyl ester cross-linking in grasses by about 50%.

Unlignified walls were rapidly and completely digested within 8-h by rumen microorganisms. Synthetic lignification of cell walls reduced the specific rate of digestion by 41% and the extent of digestion by 14% (Table 2). Reducing total lignin-feruloyl ester cross-links by 50% had no effect on the rate or extent of cell-wall digestion.

Table 1. Yields of syringyl (S) and guaiacyl (G) compounds recovered after solvolytic degradation of cell-wall lignins by thioacidolysis.

Corn lignin	Monomer			Dimers				
	Yield	S/G	Yield	5-5	β-5	β-1	5-O-4	β-β
	μM g^{-1}		μM g^{-1}	Proportion of linkage types				
Synthetic	727	1.1	80	10	30	27	22	11
Natural	593	1.4	64	11	29	20	20	21

Table 2. Hydroxycinnamic acid composition and digestion kinetics of cell walls from suspension cultured corn. Cultures were grown with 2-aminoindan-2-phosphonic acid (AIP) to reduce the deposition of ferulic acid into cell walls. Isolated cell walls were synthetically lignified by the peroxidasic polymerization of hydroxycinnamyl alcohols (HCA).

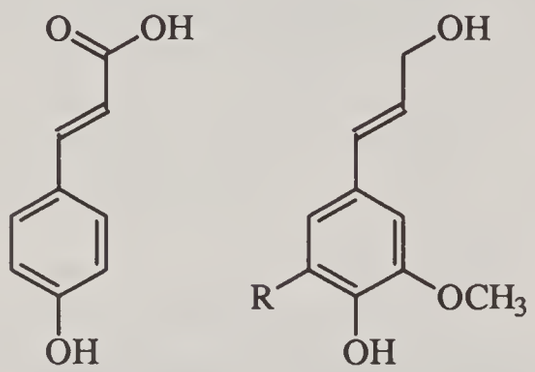
Treatment		Ferulic acid		Total Dehydrodiferulic acids		Digestion kinetics	
AIP	HCA	esters	ethers	esters	ethers	rate	extent
		mg/g cell wall				per h	mL gas
0	0	10.4	0.1	7.6	0.3	0.25	29.2
0	+	1.1	3.2	1.9	4.1	0.14	25.1
+	0	3.8	0.1	4.3	0.2	0.24	29.0
+	+	0.2	1.4	0.2	2.0	0.16	24.9

Deposition of Syringyl Lignin and *p*-Coumaric Acid in Maize Internodes

H.G. Jung, T.A. Morrison and D. R. Buxton

Introduction

Work in tree species and rice has shown that guaiacyl-type lignin is deposited early during lignification of plant cell walls and localized in the middle lamella/primary cell wall region. As lignification proceeds into the secondary wall, the deposited lignin becomes progressively richer in syringyl-type lignin. *p*-Coumaric acid (I) addition to the cell wall seems to follow a similar pattern with the majority of the *p*-coumarate being deposited during secondary wall lignification.



I II R=OCH_3 III R=H

Members of the Cell Wall Group have recently shown that *p*-coumaric acid is esterified exclusively to maize lignin at the γ-position of lignin units. This result indicates that synthesis of *p*-coumarate esters of monolignols must occur intracellularly. Furthermore, synthetic work suggests that the monolignol is probably sinapyl alcohol (II), the syringyl lignin precursor, rather than the guaiacyl lignin monolignol coniferyl alcohol (III). Our objective was to determine if the pattern of syringyl lignin and *p*-coumaric acid deposition in maize during maturation is synchronous.

Materials and Methods

Single-cross maize hybrid (Mo17 x B73) plants were grown in a growth chamber under a 12 h photoperiod and 28°/18°C day/night temperature regime. At the 14th leaf stage, internodes 7 through 14 from five plants were excised, divided into upper and lower halves, and freeze dried. The internodes were manually dissected into pith and rind tissue fractions, and then ground with dry ice in a mortar and pestle. Extractives were removed from samples by extracting with water at 90°C (2X) and acetone. Approximately 100 µg samples of each internode tissue fraction were pyrolyzed and lignin degradation products identified by GC-MS analysis. *p*-Coumaric acid was estimated by 4-vinylphenol yield, and syringyl and guaiacyl lignins were quantified by summation of six syringyl and five guaiacyl products of lignin pyrolysis. Lignin composition was estimated as the syringyl-to-guaiacyl ratio normalized for guaiacol yield of each sample. The ratio of *p*-coumarate-to-syringyl lignin was calculated as the total ion abundance for 4-vinylphenol divided by the ion abundance of the six syringyl-lignin derived products. The experiment was replicated with two sets of plants from separate growth periods.

Results and Discussion

Internode 7 was the first elongated stem internode above the soil surface and was the oldest internode sampled. Age of the internode segments decreased as position moved up the plant. The youngest internodes (12 through 14) were still actively elongating at harvest. As can be seen in Fig. 1, the composition of lignin in maize internodes changed with age of the internodes. The older internodes contained progressively more syringyl-type

lignin. Internodes 13 and 14 contained no measurable lignin as determined by the pyrolysis-GC-MS procedure. In maize stem development, the upper portion of an internode is older than the lower section, and we observed an increased ($P<0.10$) syringyl-to-guaiacyl ratio in the upper half of the sampled internodes (1.77 vs. 1.55). The more highly lignified rind tissue had a greater ($P<0.05$) syringyl-to-guaiacyl ratio than did the pith fraction (3.28 vs. 1.80) in internode seven, but the tissues did not differ ($P>0.10$) for the other internodes.

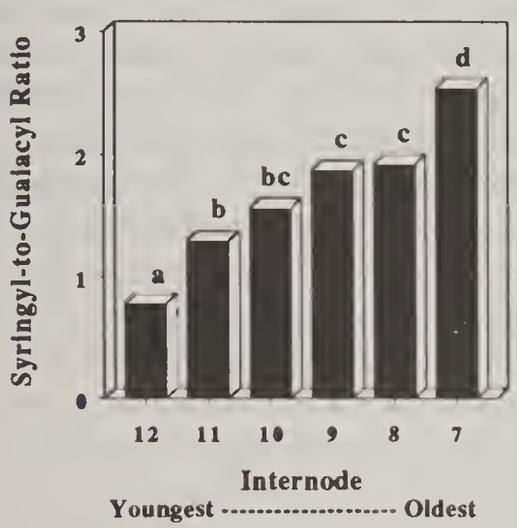


Figure 1. Lignin composition of maize internodes of different ages.

If *p*-coumaric acid esters of lignin are only deposited in conjunction with syringyl-type lignin, then we hypothesize that the *p*-coumaric acid-to-syringyl lignin ratio should be constant in all lignified tissues, regardless of the syringyl-to-guaiacyl ratio of the lignin present. This was observed to be the case for Internodes 7 through 11 (Fig. 2). The *p*-coumaric acid-to-syringyl lignin ratio in Internode 12 was elevated ($P<0.05$) compared to the others, but this may represent carbohydrate esters of *p*-coumarate rather than esters to lignin. Other work by our group has shown that *p*-coumaric acid concentration in the cell wall is closely correlated to the amount of

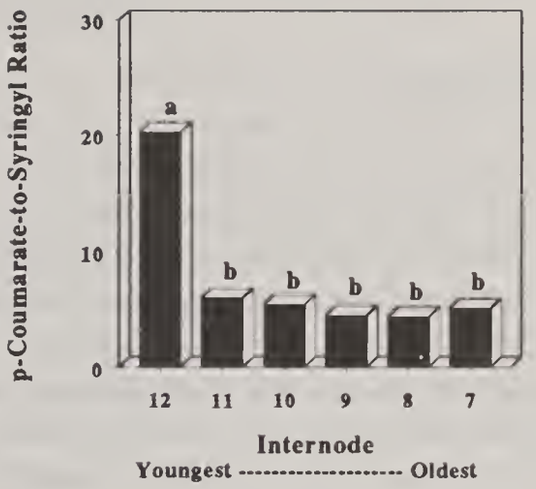


Figure 2. Ratio of p-*coumaric acid-to-syringyl lignin in maize internodes of varying age.*

lignin present, and young plant tissues, such as Internode 12, have very little lignin present. The *p*-coumaric acid esters present in poorly lignified tissues are likely to be esters to arabinoxylan in the primary cell wall. We believe that the high *p*-coumaric acid-to-syringyl lignin ratio in Internode 12 represents the *p*-coumarate that was deposited in the primary wall as carbohydrate esters rather than esters to syringyl-lignin, which is low in these young tissues.

Conclusion

The data for maize internodes agree with the hypothesis that *p*-coumarate esters of lignin are deposited in conjunction with syringyl-type lignin. We know that C_4 grasses such as maize deposit much higher concentrations of *p*-coumarate esters in the cell wall than are seen for C_3 grasses or legumes. The function of these high concentrations of *p*-coumaric acid in the cell wall of maize remains unknown.

Synthetic Dehydrogenative Polymerization for Modeling Grass Cell Wall Lignification

S. Quideau, J. Ralph and J.H. Grabber

Introduction

Dehydrogenation polymers or DHPs are synthetic lignins obtained biomimetically by one-electron oxidation of propenylphenol lignin monomers in the presence of peroxidases and hydrogen peroxide or laccases and oxygen. Resonance-stabilized phenoxy radicals are produced from the initial phenolic monomers and from phenolic intermediate oligomers, and these couple in a variety of ways to build up a polymeric structure in a manner similar to the one occurring *in vivo*. The most often cited drawback of such a lignification modeling technique is its inability to duplicate the structure of lignin isolates. Relatively low molecular mass materials and structures not detected from analyses of lignin isolates are often formed during *in vitro* dehydrogenative polymerization. However, the heterogeneity of the phenylpropanoid lignin polymer with respect not only to its monomeric composition, but also to the chemical, temporal and morphological aspects of its formation are critical concepts also too often overlooked in studies that focus on lignin isolates to elucidate the structure of lignins.

Synthetic dehydrogenative lignin polymerizations are based on a constructive approach, by contrast with the destructive approach followed during lignin isolations, and provide lignin chemists not only with a lignin-like material as representative of native lignins as any commonly used lignin isolates, but also with a unique tool to elucidate the available chemical routes leading to the formation of all main lignin structures. During our research efforts directed toward the understanding of the structural role of *p*-hydroxycinnamic acids in grass cell walls, we have been successfully using dehydrogenative polymerization techniques, in concert with the synthesis of low molecular mass model compounds, to determine what types of structures are chemically possible and likely to occur *in vivo* (see the 1991 Research Summaries).

The efficiency of the dehydrogenative polymerization as a lignification modeling technique became even more significant in light of our investigation on the acylation of maize lignin by *p*-coumaric acid (see the 1992 Research Summaries). We found that *p*-coumaric acid is esterified exclusively at the γ-position of the maize lignin side-chain. This strict regiochemistry implies that enzymatically controlled pre-acylation of *p*-hydroxycinnamyl alcohol lignin monomers and their subsequent incorporation into the lignin via oxidative coupling is the mechanism by which lignin acylation occurs. The dehydrogenative co-polymerization of the lignin monomer coniferyl alcohol with the pre-acylated lignin monomer coniferyl *p*-coumarate allowed us to successfully duplicate this mechanism (Fig. 1).

Method

The coniferyl alcohol/[9-^{13}C]coniferyl*p*-coumarate (9:1 ratio) DHP was prepared

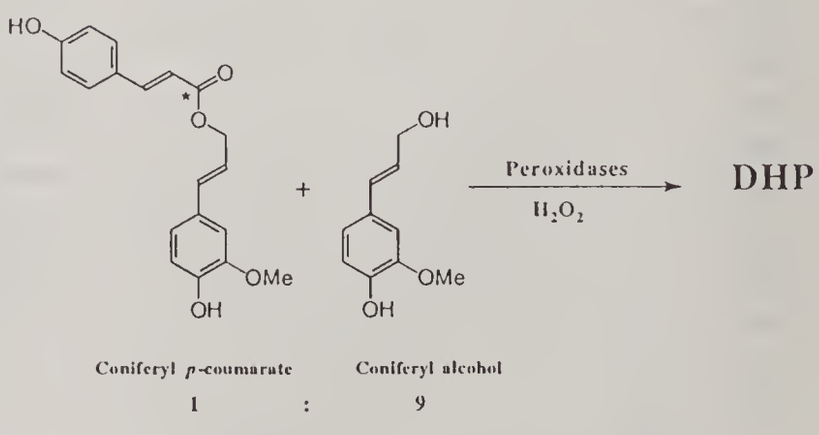


Figure 1. Modeling of the g-p-coumaroylation of maize lignin via biomimetic dehydrogenative co-polymerization of coniferyl alcohol with [9-^{13}C]-labeled coniferyl p-coumarate (9:1 ratio).

according to the standard dehydrogenative polymerization method. Coniferyl *p*-coumarate was ^{13}C-labeled at the strategic carbonyl position for NMR sensitivity enhancement purposes. The proton-detected long-range C-H correlation (HMBC) spectrum of the DHP in Fig. 2 was run on a 100 mg sample using Bruker's standard inv4lplrnd sequence with 2K data points in the proton dimension and 256 increments in the carbon dimension, using 320 scans per increment. Optimized Gaussian apodization was applied in F_2 and unshifted squared sine-bell (Q0) apodization was applied in F_1 and the matrix zero-filled and Fourier transformed (using magnitude mode phase correction) to give a final matrix of 1K by 1K real points. The maize lignin isolation procedure and NMR experimental methods were described in the 1992 Research Summaries.

lignin side-chain γ-protons. The structures of the corresponding γ-*p*-coumaroylated lignin model compounds are displayed in Fig. 3.

Although this DHP contains a large proportion of cinnamyl *p*-coumarate end-groups as compared to the maize lignin isolate (contour peak **A**, c.f. primed fragment of model **2**), the characteristic contours **B**-**D** from the other γ-*p*-coumaroylated lignin units indicate that this DHP has successfully modeled the acylated maize lignin polymer. The contour peak **B** derives from correlations in phenylcoumaran structures (c.f. model **2**), the contour peak **C** from *erythro* and *threo* β-aryl ethers (c.f. models **1**) and phenylcoumarans, and the contour peak **D** from *threo* β-aryl ethers (c.f. model **1***t*). The phenylcoumarans arising from β-5 coupling of the coniferyl *p*-coumarate (at

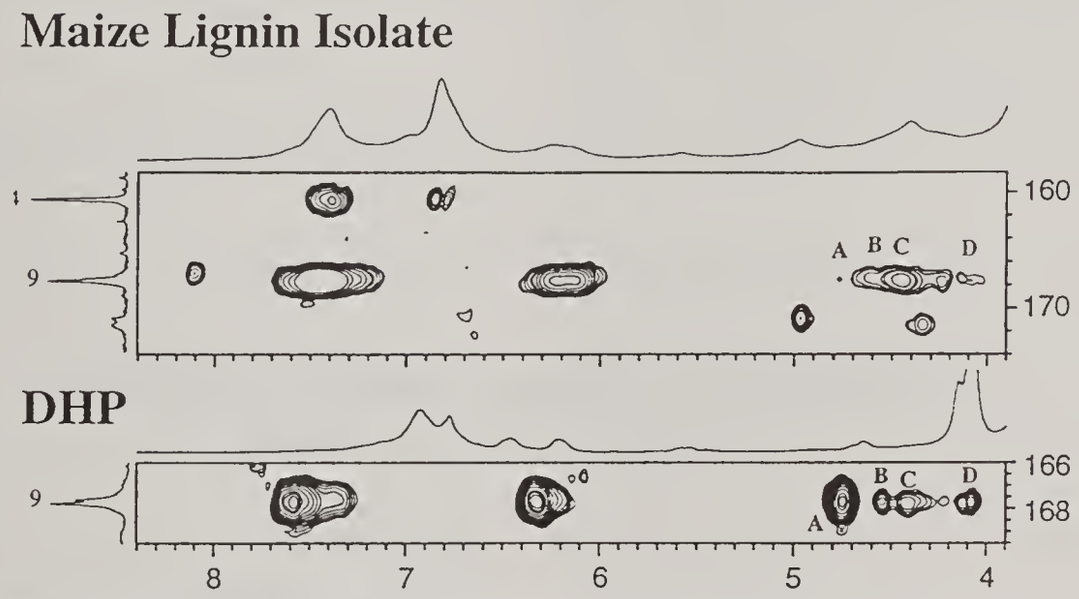


Figure 2. Carbonyl correlation sections of proton-detected long-range C-H correlation spectra of maize lignin isolate and coniferyl alcohol/coniferyl p-coumarate dehydrogenation polymer.

Discussion

The HMBC experiment correlates protons and carbons separated by two or three bonds. Figure 2 shows the section around the *p*-coumaroyl carbonyl 9-carbons of the HMBC spectrum of the maize lignin isolate, along with the equivalent coniferyl alcohol/coniferyl *p*-coumarate DHP spectral section. The contour cluster labeled **A**-**D** indicates correlations between the ester carbonyl 9-carbons and the β) with lignin monomers or oligomers (at 5) is a predominant structure (contour peak **B**) and is more distinct than that in the maize lignin isolate. High proportions of phenylcoumarans typify oxidative dimerization of (*E*)-propenylphenols and early lignification. The proportion of the *threo*-β-aryl ethers (region **D**) is obviously significantly higher than in the maize lignin. This suggests that, unlike the synthetic DHP made here from coniferyl alcohol and coniferyl *p*-coumarate, sinapyl *p*-

coumarate may also be involved in maize lignification, for it has been established that arylglycerol-β-syringyl ethers are formed predominantly in their *erythro*-forms, which have contour peaks in region C.

Conclusion

This dehydrogenative polymerization allowed us to confirm the chemical feasibility of the lignin acylation mechanism which implicates involvement of pre-*p*-coumaroylated lignin monomers during the lignification of maize cell walls. Furthermore, the comparative NMR analysis between this synthetic lignin model and the maize lignin isolate has opened new routes of investigation, notably concerning the possibility of the participation of sinapyl *p*-coumarate rather than, or in addition to, coniferyl *p*-coumarate in the lignification of C4 grass cell walls.

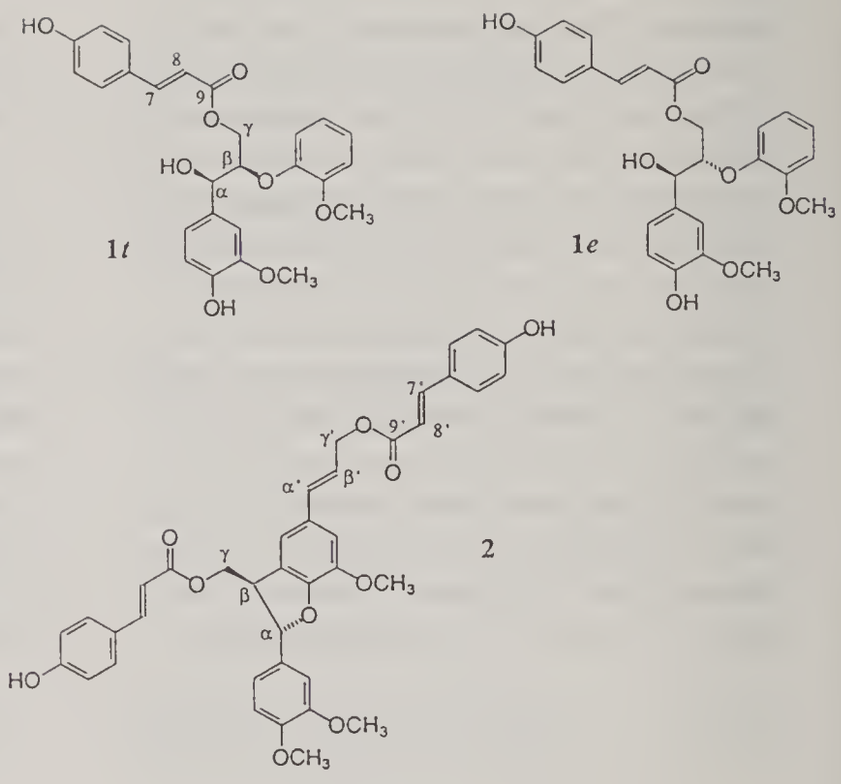


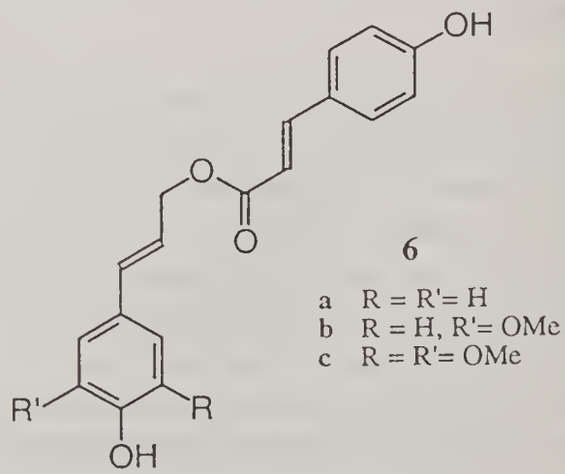
Figure 3. Synthesized g-p-coumaroylated lignin model compounds.

Synthesis of *p*-Hydroxycinnamyl *p*-Coumarates

S. Quideau, J. Ralph and J. H. Grabber

Introduction

Grasses contain significant amounts of *p*-coumaric acid **3** esterified to the lignin polymer. Investigations into the regiochemistry of *p*-coumaric acid on maize lignins led us to conclude that lignin acylation occurs by a mechanism involving participation of pre-*p*-coumaroylated *p*-hydroxycinnamyl alcohols in the lignification process. Thus, *p*-hydroxycinnamyl *p*-coumarates could potentially act as lignin precursors and co-polymerize with the common *p*-hydroxycinnamyl alcohol lignin monomers via peroxidase-catalyzed phenol oxidative coupling to give acylated lignins. The need for a facile, high-yielding preparation of *p*-hydroxycinnamyl *p*-coumarates such as coniferyl **6b** and sinapyl **6c** *p*-coumarates became compulsory. The



6

a R = R'= H
b R = H, R'= OMe
c R = R'= OMe

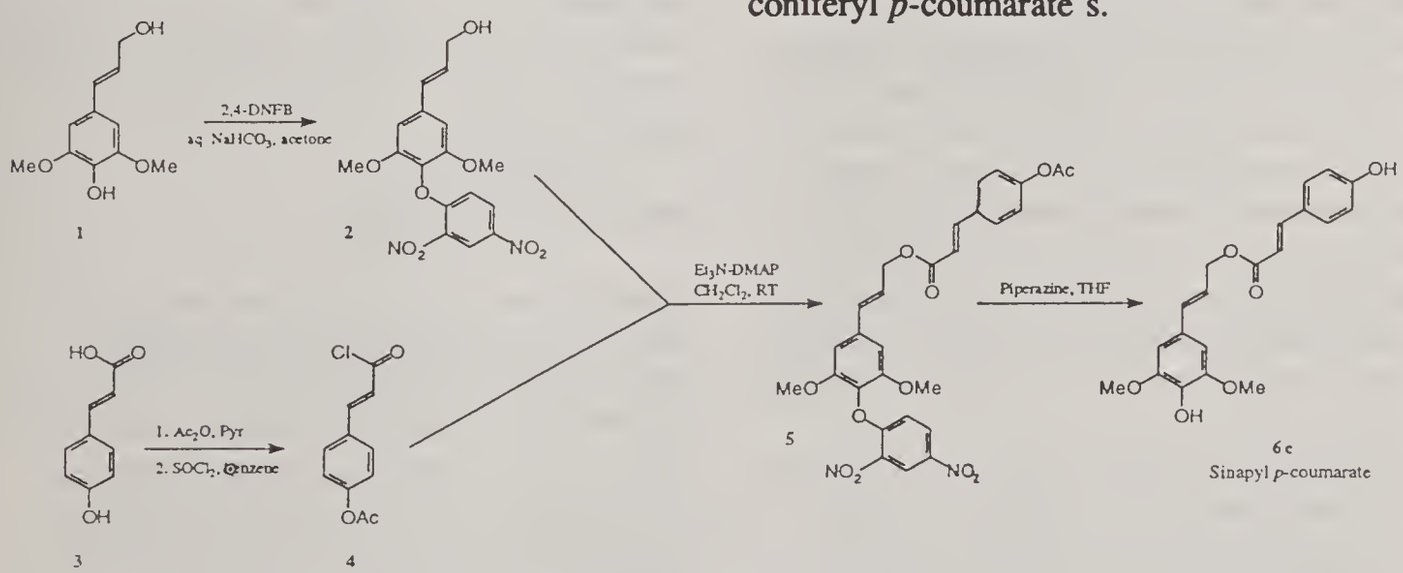
general synthetic approach we have chosen is based on a scheme proposed by Nakamura and Higuchi in 1978 for the synthesis of coniferyl *p*-hydroxybenzoate and *p*-coumarate. Modifications of this original scheme and the easy access to *p*-hydroxycinnamyl alcohols (see the 1991 Research Summaries) allowed us to develop an improved synthesis of *p*-hydroxycinnamyl *p*-coumarates.

Method

Scheme 1 displays the synthetic route to sinapyl *p*-coumarate **6c**. Coniferyl *p*-coumarate **6b** was similarly prepared. If deemed necessary, the preparation of *p*-hydroxycinnamyl *p*-coumarate **6a** could be prepared according to the same methodology. Thus, the phenolic hydroxyl group of sinapyl alcohol **1** was selectively protected by treatment with 1 equiv. of 2,4-dinitrofluorobenzene (2,4-DNFB) in the presence of an excess of aq. sodium bicarbonate to afford the 2,4-dinitrophenyl ether **2** in yields > 90%. The crystalline acid chloride **4** was obtained almost quantitatively by acetylation of *p*-coumaric acid **3** followed by treatment with thionyl chloride in refluxing benzene and recrystallization from toluene. Acylation of the 2,4-dinitrophenyl ether **2** (1 equiv.) with the acid chloride **4** (1.2 equiv.) was performed by using 1.3 equiv. of dimethylaminopyridine (DMAP) at room temperature for 1h. to give **5** in 93% yield. Removal of both protecting groups was then accomplished in one step by treatment with a large excess (ca. 10 equiv.) of piperazine in anhydrous THF at room temperature for 30 min. to afford sinapyl *p*-coumarate **6c** in 90% yield after purification through deactivated silica gel. Coniferyl *p*-coumarate **6b** was obtained in similar yields.

Discussion

A facile synthesis of *p*-hydroxycinnamyl *p*-coumarates **6a-c** was needed because of the possibility of these molecules to act as precursors of γ-acylated lignins in C_4 grass cell walls. The proposed synthetic scheme provides rapid access to *p*-hydroxycinnamyl *p*-coumarates. Notably, the use of 4-acetoxycinnamic acid chloride as acylating agent and the use of DMAP to mediate an efficient acylation make this synthetic route to coniferyl and sinapyl *p*-coumarates high-yielding and easy to follow. The choice of anhydrous THF as solvent in a room temperature piperazine-mediated deprotecting final step constitutes a further improvement over the refluxing benzene piperazine treatment proposed by Nakamura and Higuchi. The milder conditions applied here ensure the high reproducibility of the method, especially in the case of sinapyl *p*-coumarate whose sensitivity toward hydrolysis is higher than coniferyl *p*-coumarate's.



Scheme 1. Preparation of sinapyl p-coumarate - Synthetic route to p-hydroxycinnamyl p-coumarates.

Release of a Plant Cell Wall Compound NMR Database

J. Ralph, W.L. Landucci, S.A. Ralph and L.L. Landucci

Introduction

Five years ago personnel at the US Dairy Forage Research Center and the US Forest Products Laboratory decided that an NMR database of plant cell wall model compounds, with particular emphasis on lignin model compounds, would be created. Because of the agreement between chemical shifts of carbons in good model compounds and in the polymer itself, assignments in the complex lignin polymer have been substantially made from model compound data. However, lignin spectra are run in a variety of solvents, including DMSO and 9:1 acetone:water for underivatized lignins, and $CDCl_3$ and acetone for acetylated lignins. For that reason, and because ^{13}C chemical shifts can vary with solvent, it was decided from the outset that all compounds would be run in the three solvents, acetone-d_6, $CDCl_3$, and DMSO-d_6, and under a fixed set of conditions.

Implementation

Most spectra have been run routinely and assigned by reference to the literature and by comparison with related compounds in this database, but we couldn't resist fully authenticating some of the more interesting models by the usual complement of 2D experiments — it is noted in the database when fully unambiguous authentication has been performed. The result is that the database currently has some 200 compounds, and a rather large number simply entered from literature data. Some of these compounds and data are in advance of the literature; for example, the latest cell-wall cross-linking structures that have been synthesized in these laboratories are already included.

The database format itself underwent considerable development. We wanted a computer-based interactive system with considerable flexibility and some chemical intelligence. At the time, databases were considerably less sophisticated than some of the emerging ones, and our choice at the time was to write it in Apple's HyperCard, to which anyone with a Macintosh has access. That turned out to be an excellent choice from the point of view of flexibility and customizability and is now particularly open to interaction with other software through Applescript. We retain that as our favored format but have ported the data to Claris FileMaker Pro, a cross-platform database application for both MS Windows and Mac OS.

There are various viewing modes depending on whether you want to see intensities or not, proton data as well as ^{13}C data, or a line graph of the spectra. Being in HyperCard and on a wholly object-oriented platform, the interaction possibilities with other applications (e.g. molecular modeling, spreadsheets) are endless. Obviously the database features the ability to search and sort by a variety of means, including directly from the structure itself; the data can be exported in a variety of forms; there is a direct connection with chemistry drawing software; and there are many more features than can be described here and many more that continue to evolve. We shall be looking to improve interaction with chemistry database software (usually integrated with molecular modeling, etc.) to allow versatile sub-structure searching which will become important as the database grows.

Release and Availability

The database is about to be released to the general public. Until we add a little more functionality to the FileMaker Pro version, and address a few of the cross-platform issues in 1994, it will be available only in HyperCard format and on the Internet. Users wishing to access our machine via FTP on the Internet should send E-Mail to

RALPHJ@MACC.WISC.EDU so that they can be given access to our server (nmrquadra.dfrc.wisc.edu, IP# 144.92.132.68). Documentation is provided in a platform independent format. We shall be looking further into internet documentation and software resources such as NCSA Mosaic which is available now for the Macintosh and UNIX machines but not yet for DOS/Windows. The full suite of raw NMR data will also be available to selected individuals — the data remain in a format where it can be processed by Bruker NMR software as well as a number of third party processing applications. In the future we will be able to provide copies of the database and documentation on diskette for either platform and will provide hard copy versions as required, but there may be a cost associated with these distribution methods. The Internet method will provide access to the continually changing database and is the preferred distribution. There will also be instructions for researchers to add compounds to their own database and to submit those entries (via an export feature) to us for inclusion in the official database. Data in this database can be cited in publications — this is a repository for data that will be available in the long term. We expect this database to grow substantially and to evolve into a complete tool for researchers in the plant cell wall chemistry area.

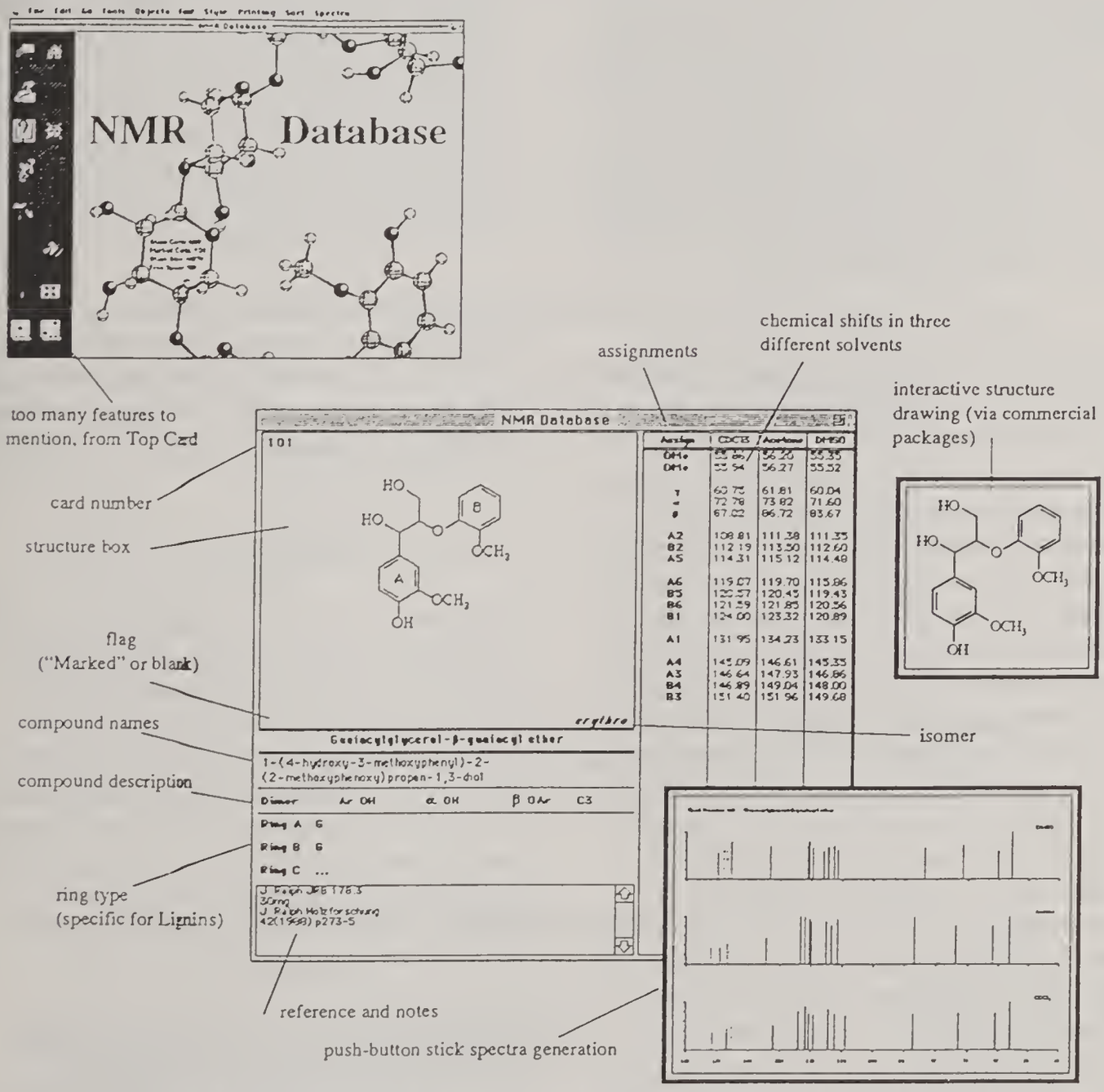


Figure 1. Composite figure showing a sample card views and a few of the features.

Effects of Chemical and Physical Treatments on the Crystallinity of Cellulose

P.J. Weimer, J.M. Hackney and J.M. Lopez-Guisa

Introduction

Cellulose is unusual among polysaccharides in that individual chains of its component monosaccharides are typically arranged in a spatially ordered manner through intrachain and interchain hydrogen bonding. In natural cellulosic materials, these crystalline regions are generally dispersed in a random and continuous fashion throughout the fiber and crystallinity is normally quantified by the so-called "relative crystallinity index" (RCI). Because different methods for measuring RCI yield different values for the same substrate, RCI values do not truly quantitate the fraction of glucosyl units that are hydrogen-bonded into a crystalline array. Nevertheless, RCI values are useful for rank ordering the crystallinity of different celluloses, and for determining how crystallinity is affected by various physical and chemical treatments.

Crystallinity is of particular interest with regard to cellulose biodegradation. It has been reported to be an important determinant of the rate of cellulose digestion by cellulolytic microorganisms, although its effects appear to vary widely among different microbes. A number of studies have reported that residual cellulose from microbial incubations has a higher RCI than that of the original cellulose, and on this basis have suggested that amorphous regions are preferentially degraded by the microorganisms. In these studies, however, the measurements of RCI were normally conducted under conditions which may have allowed cellulose to recrystallize. Consequently, the actual crystallinity of the substrates in the incubation medium (i.e., in contact with the microbe or its cellulases) may have been substantially lower, and the conclusion that the amorphous regions are more rapidly degraded may be untenable. The purpose of this study was to systematically examine how crystallinity is affected by various treatments commonly used in recovery of cellulose from microbial cultures.

Materials and Methods

Two commercial celluloses (Sigma, St. Louis, MO) were used: SC50 microcrystalline cellulose, and SC100, a cellulose of somewhat lower crystallinity. The celluloses were subjected to the following treatments: boiling neutral detergent (60 min); boiling acid detergent (60 min); alkaline hydrogen peroxide (1.5 % w/v, pH 11.5, room temperature, 6 h); acid chlorite (5 hourly additions of Na chlorite[0.14% w/v] and glacial acetic acid [0.07% w/v], 70°C); rehydration and autoclaving (30 min, 124°C); dry autoclaving (30 min, 124°C); rehydration without autoclaving. Each sample was thoroughly washed after treatment and was then frozen and lyophilized.

The relative crystallinity indices of dried materials were determined by x-ray diffraction (CuK α radiation) in both the transmission and reflectance modes. RCI of samples in aqueous media were determined by kinetics of hydrolysis in boiling 6 N HCl.

Results and Discussion

Treatment with various reagents often used for forage fiber analysis, followed by lyophilization, caused only small increases in RCI for microcrystalline cellulose SC50, but larger increases for the less crystalline SC100. The extent of recrystallization was relatively minor for treatments conducted at ambient temperature, but increased with the temperature of the treatment. Even simple resuspension of SC100 in water followed by lyophilization resulted in a detectable increase in RCI.

To examine the effects of temperature and temperature cycling on recrystallization, SC100 was suspended in water and subjected

to different temperatures (50, 75, 99, or 124°C) and different numbers of heating cycles (1, 2, or 3). After equilibration to room temperature, each sample was lyophilized and its RCI measured by x-ray diffraction. The data revealed: i) extensive recrystallization at 50°C, with little additional increase in RCI at higher temperatures; and ii) no further increase in RCI upon additional stages of heating. Dry autoclaved samples subjected to similar treatments showed only minor increases in RCI, suggesting that water availability stimulates recrystallization.

To determine the relative contributions of rehydration and heating to the recrystallization process, SC100 was subjected to four different combinations of heating and rehydration (Table 2). In each case, HCl was added to a concentration of 6 N thirty minutes after the final resuspension, and the RCI determined by acid-hydrolysis kinetics on the fully hydrated samples, without the necessity of a drying step needed for x-ray diffraction analysis.

The data suggest that the lyophilization (drying) step causes only a slight increase in RCI relative to rehydrated materials. It is therefore likely that recrystallization is an unavoidable consequence of rehydration.

Conclusions

The crystallinity of pure cellulose increases substantially upon rehydration in aqueous media. Further recrystallization occurs during heating or drying steps used for recovery of celluloses for crystallinity analysis by x-ray diffraction. As a result, increases in crystallinity reported by others to result from preferential microbial attack on amorphous regions may simply be artifacts of the heating or drying process used to recover the cellulose from the microbial cultures. To minimize these artifacts, comparison of RCI of celluloses before and after microbial attack should include control incubations in which the original celluloses are carried through the same treatments in the absence of microorganisms.

Table 1. Effect of various chemical and physical treatments on the crystallinity of celluloses SC50 and SC100

	Relative Crystallinity Index[b]	
Treatment[a]	SC50	SC100
None (as-received commercial material)	83.2	76.2
Rewetting	85.9	79.8
Alkaline hydrogen peroxide (AHP)	84.8	78.3
AHP/Acid chlorite (AHP/AC)	83.6	80.4
Neutral detergent (ND)	84.5	81.8
Acid detergent (AD)	84.8	82.3
ND / AD / AHP / AC	85.2	84.0
Wet autoclaving	83.8	81.1

[a]See Materials and Methods for details of treatments. Following all treatments that involved rehydration in aqueous media, the celluloses were thoroughly rinsed and then lyophilized.
[b]Determined by x-ray diffraction in the reflectance mode. Results are mean values of two x-ray diffraction runs.

Table 2. Differential effects of heating and rehydration on RCI of cellulose SC100.

	RCI (mean + S.E.M.)
Resuspend dry commercial powder in water at 25°C	59.7 ± 0.3
Resuspend in water, lyophilize, resuspend in water	62.4 ± 0.9
Resuspend in water, heat to boiling	67.4 ± 1.0
Resuspend in water, heat to boiling, lyophilize, resuspend in water	68.5 ± 1.1

Cellulose/xylan Composite Structures for the Study of Plant Cell Wall Digestion

P.J. Weimer, J.M. Hackney, C.R. Dietrich and H.G. Jung

Introduction

Plant cell walls are composed of a number of biopolymers (cellulose, various hemicelluloses, lignin, and proteins) whose arrangement is both complex and variable with tissue type. Digestion of plant cell walls by ruminal microbes and their hydrolytic enzymes is thought to be limited by a number of factors, primarily the sequestering effects of lignin and the presence of certain covalent linkages (e.g., between phenolic acids and arabinoxylans).

Another potential determinant of plant cell wall digestibility is the interactions among the polysaccharide components themselves. These interactions have received little study, perhaps due to the large number of biopolymers that can potentially interact with one another and by structural variations within individual cell types. However, these interactions may be studied more systematically by preparing synthetic composite materials of defined polysaccharide compositions and by characterizing these materials with respect to their structure and biodegradation behavior. While these materials would be simpler in structure than would authentic plant cell walls, they should display some of the same types of chemical interactions and thus may serve as simple model systems for evaluating the effects of certain types of interactions (e.g., hydrogen bonding, chain intercalation and cellulose crystallinity) on digestion kinetics. As a first step toward this goal, we have prepared and characterized several types of composite materials containing cellulose and xylan, the two major polysaccharides in plant cell walls.

Materials and Methods

Tobacco xylan was isolated by solvent extraction of flue-cured tobacco stalks, followed by acid chlorite delignification of the unextracted solids and alkaline extraction of the delignified holocellulose. The alkaline extracts were neutralized, precipitated with 80% ethanol, and dialyzed against water prior to freeze drying. The isolated xylan contained ~8% uronic acids and 92% neutral sugars with xylose representing ~ 95 % of the neutral sugar content. Xylose was almost exclusively in the β-1,4-linkage, and the material was highly water-soluble despite an average DP of 88.

Biosynthetic composites were prepared by growing the cellulose-synthesizing bacterium *Acetobacter aceti* var. *xylinum* in glucose-containing Hestrin and Schramm medium supplemented with a linear, water-soluble homoxylan isolated from tobacco stalks. The composites were purified by solvent extraction, acid chlorite bleaching and boiling in alkali. The final product was exhaustively dialyzed against water prior to freeze drying.

Chemisynthetic composites were prepared by first performing an Isogai-Atalla dissolution of the cellulose in a mixture of dimethylsulfoxide, sulfur dioxide, and diethylamine. The cellulose was then regenerated in water in the presence of an equal weight of tobacco stalk xylan, or was mixed with the tobacco xylan to form a slurry, which was then reprecipitated by addition to pure water. In both cases, the regenerated gelid composites were broken apart in a Waring blender, exhaustively dialyzed against water, and lyophilized.

Results and Discussion

In the *Acetobacter aceti* cultures the biosynthetic composites were tan and filamentous in appearance, in contrast to the white spherules of pure cellulose produced by the organism when growing on glucose alone. X-ray diffraction [Fig.1] revealed that these composites were highly crystalline (although somewhat less so than pure bacterial cellulose), and

chemical analysis indicated that the composites had maximal levels of xylan incorporation of approximately 9% (molar basis).

The chemisynthetic composites prepared by regeneration of dissolved cellulose into an aqueous solution of xylan had maximal levels of xylan incorporation of approximately 7% (molar basis).

By contrast, the composite prepared by regenerating the dissolved cellulose/insoluble xylan slurry in pure water contained up to 40% xylan, although this xylan was probably not distributed randomly in the final product. X-ray diffraction revealed that the chemisynthetic composites were essentially noncrystalline. Levels of xylan incorporation into the composites increased with increasing concentrations of xylan in the medium.

Conclusions

Biosynthetic and chemisynthetic composites of cellulose and xylan have been prepared and partially characterized. These materials provide a series of composite structures having different compositions and crystallinities and, thus, will be useful in evaluating the role of interactions between cellulose and xylan on the ruminal digestion process.

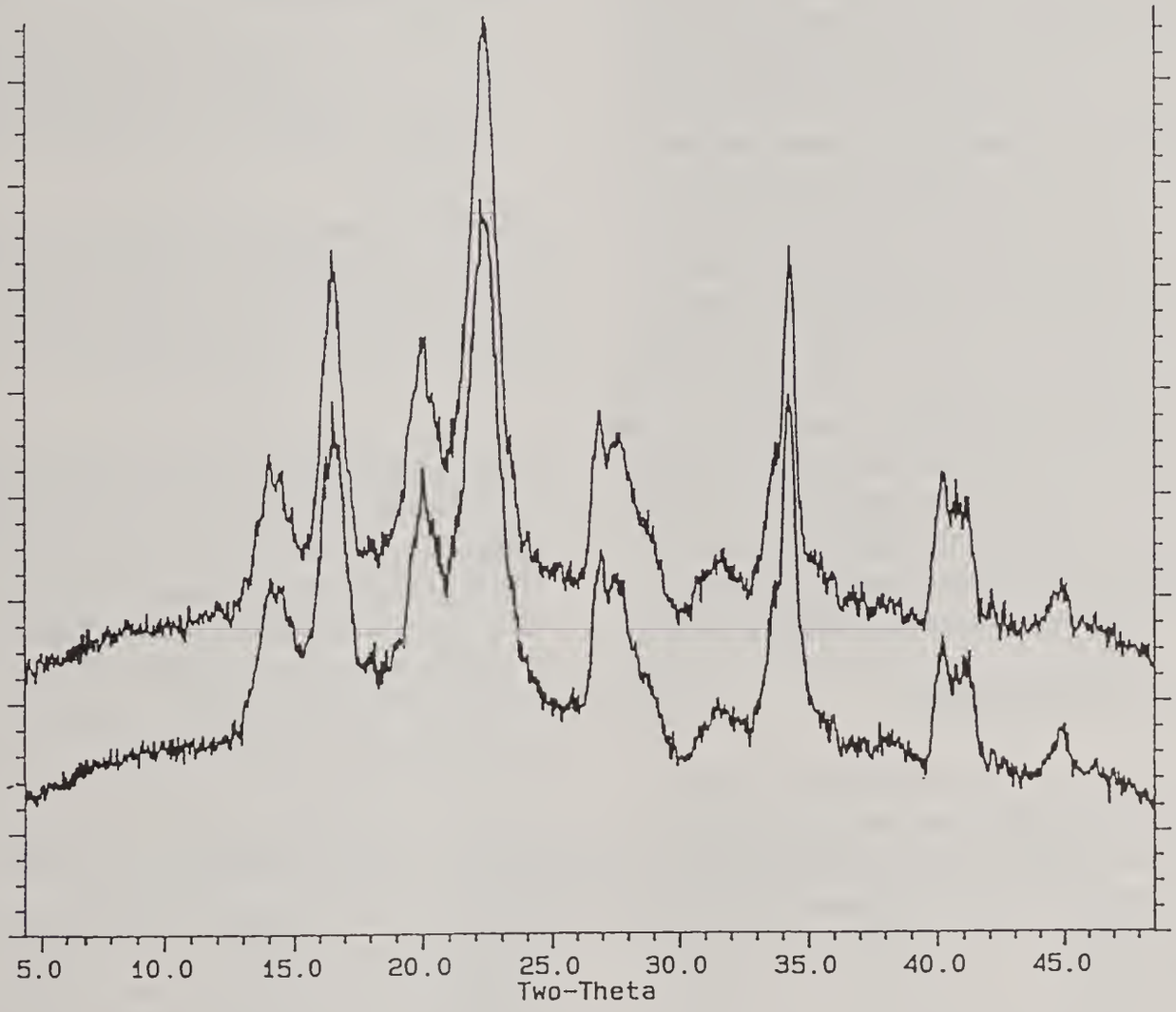


Figure 1. X-ray diffractograms of cellulose produced by A. aceti var. xylinum during growth on glucose (top), and of a composite produced by the same organism growth on glucose in the presence of 0.5% xylan (bottom); this composite contained approximately 9.2% xylan. The higher valley in the amorphous region (20 = 18°) and the slightly broader width-at-half-height in the 120 (20°) and 020 (22.5°) lattice diffractions of the composite indicate a slightly lower average crystallinity. Calculated relative crystallinity indices for the pure cellulose and the composite were 92.2 and 88.4, respectively.

Estimating Pectins in Forage Legume Samples

R.D. Hatfield, K. Brei and R.R Smith

Introduction

The full potential of the high protein content in alfalfa is not realized due to rapid degradation rates and conversion to ammonia in the rumen, leading to increased nitrogen excretion. A potential strategy to improve utilization of plant protein involves matching the rapidly degraded protein with an equally rapidly degraded carbohydrate source such that energy would not be limiting to rumen microbes. This assures that the plant protein would be efficiently converted to microbial biomass (including protein) benefiting the ruminant. Pectic polysaccharides represent a potential source of rapidly degradable carbohydrate without hindering rumen function. A significant portion of the total structural polysaccharides in alfalfa cell walls (CW) are pectins, and selection for genotypes with increased pectin content would result in improved utilization of its nutritional potential. However, the genetic diversity for pectic polysaccharides in alfalfa is not known due to the difficulty in assessing plant materials for pectins. For this reason we have undertaken a project to develop a straightforward and relatively simple method to quantify total pectic material in forage legumes.

Materials and Methods

Alfalfa samples used for the development of the pectin determination procedure (PDP) were field grown plants of various genetic backgrounds. The major steps of the PDP are shown in Fig. 1. Each step will be described briefly.

Sample preparation: Dry samples (150-200 mg) are weighed into 50 mL capped centrifuge tubes, extracted with 25 mL of 80% EtOH by sonicating for 10 min and pelleting the residue by centrifugation before decanting the supernatant. This extraction sequence is repeated 4 times with 80% EtOH, once with chloroform:methanol (2:1 v/v), and 2-3 times with acetone before air drying overnight followed by oven drying at 50-60°C overnight.

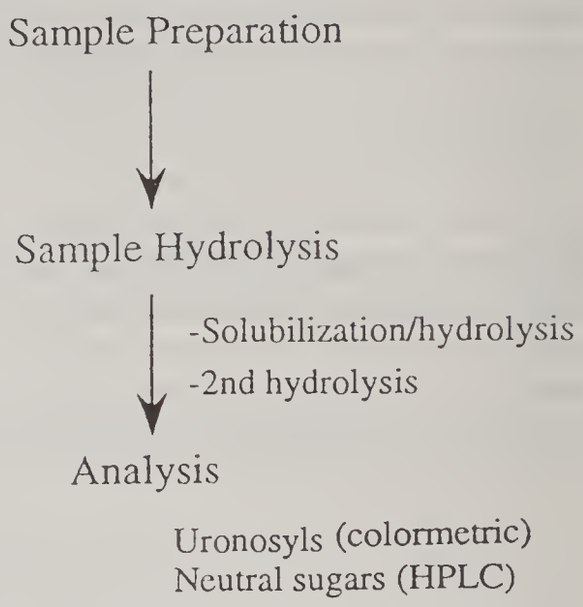


Figure 1. Flowchart of pectin determination procedure.

Sample hydrolysis: Subsamples (20-25 mg) are weighed into 8 mL vials, 3 glass beads added to each along with 500 µL of 12M H_2SO_4. Vials are capped and allowed to solublize/hydrolyze at room temperature for 2 h with vortexing every 10-20 min. The acid is diluted with 3.5 mL of H_2O, mixed, and centrifuged (500 x g) for 10 min. An aliquot (100 µL) is removed for uronosyl analysis before remixing the samples and hydrolyzing at 100°C for 3 h. Samples are cooled, internal standard added (2-deoxy-D-glucose), then filtered through glass fiber filters to collect the insoluble residue which is extensively washed with H_2O. The original hydrozate and washes are combined, mixed, and 10 mL removed and neutralized with $BaCO_3$. Insoluble $BaSO_4$ is removed by centrifugation and filtering through glass fiber filters before passing the filtrate sequentially through cation and anion exchange columns.

Analysis: Subsamples (100 µL) removed before the secondary hydrolysis step are diluted to 1 mL with H_2O and analyzed for total uronosyls using the 3-phenyl phenol method. Neutralized cell wall hydrozates are analyzed for neutral sugar composition using the Dionex HPLC system.

Discussion

Our aim was to minimize the number of steps normally required for quantitative CW analysis. However, initial trials indicated that a pre-extraction was necessary to remove cytoplasmic contaminates that interfered with the colorimetric assays and gave high readings for Gal, Ara, Rha. This is due to oligosaccharides as well as sugar nucleotides (UDP-Gal, etc.) that are present in the cytoplasm. This adds several steps to the total procedure but provides more reliable CW information.

Total pectin values are the sum of total uronosyls (from the colorimetric assay) and Rha + Gal + Ara from the neutral sugars analysis (Dionex HPLC). A limitation of this procedure is not distinguishing among the three types of uronosyl residues found in plants - galacturonic acid (GalA), glucuronic acid (GlcA) and 4-O methyl glucuronic acid (4-O-MeGlcA). We have tried modifying the 3-phenyl phenol procedure to be specific for each type but have been successful only in eliminating the GlcA response from a mixture. The values obtained from stem samples of field grown alfalfa and red clover show some variation for pectic materials (Table I). The three neutral sugars, Rha, Gal and Ara, are found only in polysaccharides that would be considered to belong to the pectic fraction based on wall fractionation of alfalfa. Values obtained by this procedure are in reasonable agreement with wall fractionation data for alfalfa stems. One should use caution in extrapolating to other forages; however, the procedure described allows a relatively rapid and quantitative method of initially screening forages for pectic enriched walls.

Although the procedure is adequate for initial screening, there are potential areas of improvement which would increase sample throughput or increase quantitative reliability (specific quantification of individual uronosyls). As data are collected, it may be possible to refine the estimates by using ratios of the individual components (i.e, Rha:uronosyls). Use of single container extraction and hydrolysis would eliminate subsampling. Using extracted polysaccharides as models, it may be possible to calculate hydrolysis/recovery efficiencies of individual uronosyls that would allow quantitation by HPLC (Dionex). This would also necessitate development of a new cleanup and analysis procedure. The information that can be obtained by the current procedure should be adequate to determine if other screening procedures, such as NIR, could be used reliably .

Table 1. A sample of data from the analysis of legume stems using the PDP.

	% of original dry matter				
Sample[a]	Uronosyls	Rha	Ara	Gal	Total
107	11.24	0.71	2.28	1.85	16.07
225	10.12	0.80	2.33	1.60	14.85
302	9.45	0.71	2.27	1.99	14.42
318	12.33	0.78	2.98	1.77	17.86
311	10.88	0.70	2.58	1.95	16.11
330	12.61	0.87	2.97	1.96	18.41

[a]Samples 107,225, 302 and 318 are from alfalfa plants. Samples 311 and 330 are red clover.

Importance of Sodium Sulfite on the Recovery and Composition of Detergent Fibers

R.W. Hintz , D.R. Mertens and K.A. Albrecht

Introduction

Sodium sulfite (Na_2SO_3) was included in the original neutral detergent fiber (NDF) method to reduce protein contamination. Loss of phenolic acids and lower lignin recoveries when sodium sulfite is used suggests that it might degrade lignin. This conclusion resulted in a modification of the NDF procedure in which sodium sulfite was removed. However, it is not clear whether lower lignin values are due primarily to loss of lignin or reduction in the protein contamination of lignin residues. The purpose of these experiments was to evaluate the effect of sodium sulfite on the recovery and composition of NDF, acid detergent fiber (ADF), and acid detergent lignin (ADL) using 72% sulfuric acid when measured sequentially on alfalfa and a diverse set of feeds.

Materials and Methods

Fiber methods were similar to those described by Goering and Van Soest (1970, USDA Agric. Handbook No. 379) with the following modifications: (1) heat-stable alpha amylase was added during refluxing and the first washing of fiber residues during NDF extraction, (2) sodium sulfite was added or eliminated, and (3) ADF and ADL were determined on the previously extracted NDF residues. Alfalfa harvested at initial growth (n = 120) and regrowth (n = 60) was analyzed in duplicate for NDF, sequential ADF (sADF), and sequential ADL (sADL) with and without the use of sulfite during NDF extraction. Twenty-four animal feeds were also analyzed for NDF, sADF, and sADL with and without the use of sodium sulfite during NDF extraction and the residues were analyzed for nitrogen using the Carlo Erba NA 1500 combustion analyzer. These 24 feeds were also analyzed for NDF using 0, 0.25, 0.5, and 1.0 g sodium sulfite per 0.5 g of sample.

Results and Discussion

Addition of 0.5 g of sodium sulfite per 0.5 g of sample consistently reduced the recovery of NDF, sADF, and sADL for the 180 alfalfa samples (Table 1). Differences between NDF extraction with or without sodium sulfite were greater for NDF (1.3 to 1.9% units) than for sADF (1.3 to 1.5% units) or for sADL (0.6 to 0.7% units). In addition, the variation between duplicate analyses was decreased when sulfite was used during NDF extraction. This confirms the research of others that the lignin values of forages (alfalfa) are reduced when sulfite is used in the NDF method.

Similarly, using sulfite decreased the NDF, sADF, and sADL values of the 24 samples selected to represent a wide range of animal feeds (Table 2). However, the magnitude of the difference between NDF values when sodium sulfite was or was not included varied dramatically among feeds. It appears that sodium sulfite must be used to obtain valid NDF values for animal products (fish meal and meat scraps), heated or cooked by-product feeds (brewer's grains and distiller's grains), and plant protein supplements (soybean meal, sunflower meal, and canola meal). The NDF values of grains and their by-products were affected very little by sulfite addition, whereas most forages had NDF values that were 1 to 2% units lower when sulfite was used in the NDF procedure. Use of sodium sulfite decreased the average variation between duplicate analyses for all methods.

Nitrogen analysis of fiber residues indicates sulfite removes significant protein contamination from the fiber residues of animal products, heated by-product feeds, and protein supplements. The removal of protein contamination was much greater for NDF than for sADF and sADL. Additions of sodium sulfite from 0 to 1.0 g per 0.5 g sample showed an exponential decline in NDF values. The greatest difference occurred between 0 and .25 g sulfite per sample with no difference in NDF values between samples extracted with 0.5 and 1.0 g sulfite per sample.

Conclusion

To obtain valid NDF values for all feeds, it is recommended that 0.5 g sodium sulfite per 0.5 g of sample should be included in the NDF procedure. Much of the difference in NDF residue weight between samples extracted with or without sulfite is nitrogenous in nature. Use of sodium sulfite in the NDF procedure will result in lower values for sequentially extracted ADF and ADL. Although some of the difference between sADF and sADL when sulfite is used during NDF extraction may be due to the loss of phenolics or lignin, a significant portion of the difference is related to protein contamination of these residues.

Table 1. Effect of sodium sulfite on the recovery of fiber and lignin residues and on variation between duplicates during sequential analysis of alfalfa forage samples.

	% NDF			% sADF			% sADL		
	Sulfite Trt.			Sulfite Trt.			Sulfite Trt.		
	Without	With	Diff.	Without	With	Diff.	Without	With	Diff.
Initial Growth (n = 120)									
Average	36.49	35.19	1.30	27.21	25.94	1.27	5.75	5.11	0.64
Variation	1.85	1.81	0.04	1.27	1.13	0.14	0.38	0.15	0.23
Regrowth (n = 60)									
Average	27.00	25.11	1.89	20.12	18.65	1.47	4.38	3.65	0.73
Variation	1.73	1.50	0.23	1.50	1.28	0.22	0.17	0.09	0.08

Table 2. Effect of sodium sulfite addition on the recovery of NDF, ADF and ADL residues and associated error variances.

		% NDF			% ADF			% ADL		
	Initial	Sulfite Trt.			Sulfite Trt.			Sulfite Trt.		
Feed	% CP	Without	With	Diff.	Without	With	Diff.	Without	With	Diff.
Fish Meal	53.94	30.44	6.27	24.16	3.78	3.72	0.06	0.39	0.24	0.15
Brewer's Grains	30.44	52.32	40.87	11.46	14.18	12.85	1.33	3.31	2.21	1.10
Distiller's Grains	25.57	38.56	27.89	10.67	8.81	7.54	1.27	1.62	1.03	0.58
Meat Scraps	52.91	30.79	22.18	8.61	6.00	3.07	2.94	1.06	0.05	1.01
Soybean Meal	46.15	18.48	12.44	6.04	8.51	7.05	1.46	0.62	0.15	0.47
Sunflower Meal	31.66	38.52	35.20	3.32	26.58	24.42	2.15	8.08	7.99	0.09
Canola Meal	40.83	23.72	20.88	2.84	14.50	14.15	0.35	5.74	5.64	0.09
Bermudagrass	10.41	67.33	64.76	2.57	34.38	32.67	1.71	5.25	3.43	1.81
Smooth Bromegrass	10.05	66.56	64.19	2.37	38.43	37.84	0.59	4.55	2.89	1.66
Switchgrass	6.00	77.61	75.90	1.70	47.71	46.24	1.47	8.58	7.69	0.88
White Clover	18.54	31.88	30.31	1.57	23.02	21.06	1.95	4.69	4.36	0.32
Alfalfa Haylage	17.06	43.57	42.15	1.42	33.63	31.94	1.69	8.73	7.87	0.86
Corn Silage	7.65	36.08	34.74	1.33	18.55	17.47	1.08	2.02	1.02	1.01
Shelled Corn	10.73	11.36	10.07	1.29	3.57	2.93	0.64	0.51	0.36	0.15
Alfalfa Hay	16.35	45.51	44.28	1.23	32.51	33.18	-0.67	6.88	6.02	0.86
Wheat Straw	3.08	80.81	79.61	1.20	49.22	47.93	1.29	6.34	4.68	1.67
Soybean Hulls	10.68	62.23	61.09	1.14	42.82	42.90	-0.07	2.47	1.75	0.73
Citrus Pulp	6.52	21.27	20.20	1.07	15.48	14.24	1.24	2.16	1.30	0.87
Barley Hay	8.27	55.78	55.33	0.45	30.34	30.56	-0.22	3.45	2.71	0.74
High-Moisture Corn	8.69	15.13	14.68	0.45	5.14	5.28	-0.13	0.68	0.23	0.45
Sheep Feces	13.56	55.11	54.76	0.35	40.85	41.21	-0.36	15.64	15.20	0.44
Wheat Midds	18.44	34.15	33.86	0.29	9.85	10.58	-0.73	3.62	2.72	0.90
Barley Grain	10.74	20.27	20.11	0.15	5.32	5.69	-0.37	0.32	0.62	-0.31
Whole Grain Oats	11.98	26.29	26.24	0.05	11.88	11.28	0.60	2.75	1.95	0.80
Average	19.59	40.99	37.42	3.57	21.88	21.07	0.80	4.14	3.42	0.72
Duplicate variation		2.19	0.96		1.97	0.26		0.26	0.22	

Red Clover Inhibits Legume Proteolysis

B.A. Jones, R.E. Muck and R.D. Hatfield

Introduction

Proteolysis of ensiled alfalfa results in the degradation of 44-87% of the forage's protein during ensiling, which translates into an economic loss for farmers with high-producing dairy cows. Interestingly, red clover, a legume of similar protein content, will have only 6-40% of its original protein degraded during ensiling. Prior research has demonstrated that the lower extent of proteolysis in red clover was not due to differences between red clover and alfalfa in total proteolytic activity or to differences in pH and temperature optima and stabilities of the proteolytic activity. The following research was undertaken to establish that red clover does have a lower extent of proteolysis during ensiling than alfalfa and to investigate what factor in red clover may cause this.

Material and Methods

Two ensiling studies were performed comparing red clover and alfalfa, one using greenhouse grown plants and the other using forages harvested from field plots. All legumes were harvested at early bud, chopped by hand (1.2 cm length), inoculated with lactic acid bacteria (10^3 live bacteria per gram herbage) and ensiled into laboratory silos. Silos were incubated at 30°C and duplicate silos were frozen at 0, 1, 3 and 7 days. Fresh herbage and silages were analyzed for dry matter (DM), pH, free amino acid nitrogen (FAA-N), ammonia nitrogen (NH_3-N) and nonprotein nitrogen (NPN), while fresh herbage was also analyzed for total nitrogen.

A separate experiment using legume leaves was performed to investigate why red clover had reduced proteolysis. Leaves of red clover and alfalfa were harvested, ground in liquid nitrogen and suspended in 50 mM $KHPO_4$ (pH 7.0, 3 ml buffer / g leaves). The slurry was filtered through cheesecloth and the filtrate was centrifuged to remove particulates (20,000 x g, 25 min). Supernatants were collected, kept on ice and used as the legume extracts. Treatments were: Trt 1, untreated alfalfa extract; Trt 2, untreated red clover extract; Trt 3, alfalfa extract with 20 mM ascorbate (Ascor) and 14 mM beta-mercaptoethanol (BME); Trt 4, red clover extract treated with 20 mM Ascor and 14 mM BME; Trt 5, untreated alfalfa and red clover extracts mixed 1:1; and Trt 6, untreated alfalfa and boiled red clover extracts mixed 1:1. The release of free amino acids over time (0 to 4 h, pH 7.0, 30°C) in treated legume extracts were determined by a ninhydrin assay after precipitation of proteins by the addition of trichloroacetic acid.

Results and Discussion

Red clover and alfalfa were ensiled at 20.6 and 16.9% DM for the greenhouse forage and 23.3 and 25.3% DM for forage from the field plots, respectively. Table 1 displays the pH and the nonprotein nitrogen composition of the fresh herbage and 7 day silages. The trends in proteolysis at 1 and 3 d of ensiling were similar to those at 7 d. The pH of the forages harvested from the greenhouse did not decline substantially with the red clover and increased with alfalfa during ensiling due to residual insecticidal soap on the forage inhibiting the growth of the lactic acid bacteria (data not shown). Legumes harvested from the field plots ensiled better than the legumes from the greenhouse as noted by the lower pH (Table 1); yet none of these legume silages attained a low pH. After 7 d of ensiling, when essentially all proteolysis has occurred, red clover silage was 40, 32 and 30% lower in the increase in NPN, FAA-N and NH_3-N than alfalfa silage, respectively. This lower extent of proteolysis could not be explained by differences in DM or by pH of the silages.

Figure 1 shows proteolysis that occurred in various treatments of alfalfa and red clover extracts. Untreated alfalfa extract (Trt 1) and alfalfa extracts treated with Ascor and BME (Trt 3) or boiled red clover extract (Trt 6) had

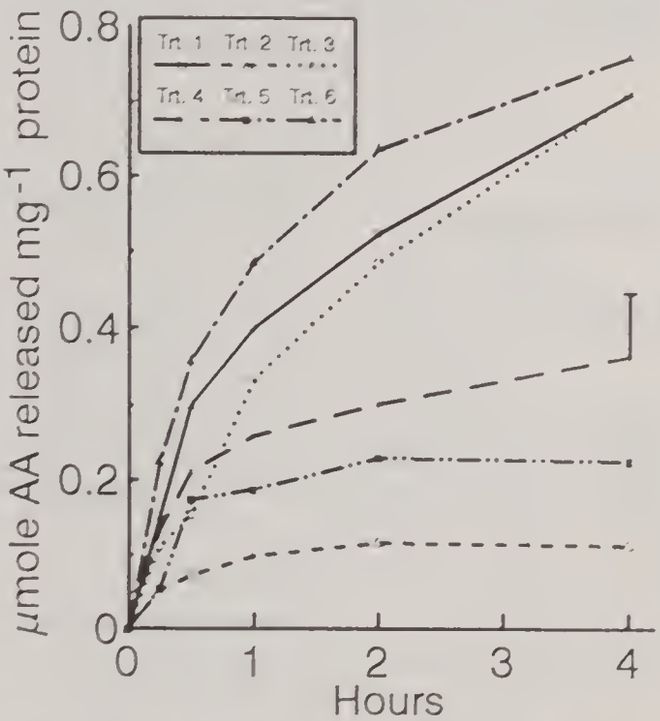


Figure 1. Proteolysis of alfalfa and red clover extracts undergoing different treatments (see Materials and Methods). Bar represents the greatest standard deviation.

the highest proteolytic activities and were not significantly different except at 0.5 h (Trt 1 and 6 were different from Trt 3). In comparison, untreated red clover (Trt 2) had the lowest activity and was significantly different from Trts 1, 3, 4 and 6 at 1 through 4 h incubations. Mixing untreated alfalfa and red clover extracts significantly reduced alfalfa proteolysis by 70% (comparing Trts 1 with 5) and was significantly lower than red clover treated with Ascor and BME (Trt 4). Yet, when boiled red clover extract was mixed with untreated alfalfa extract (Trt 6), there was no effect on alfalfa proteolysis at any time point of the incubation (comparing Trts 1 and 6).

Inactivation of the red clover factor by boiling suggests that the factor is a protein. In addition, this protein in red clover was inhibited by Ascor and BME (Trt 4), but after 0.5 h, the inhibitors were oxidized sufficiently to result in a browning of the red clover extract. The inhibition of proteolysis correlated with the presence of browning of the solutions. Inhibition of the red clover protein by Ascor and BME, along with the browning of the red clover extract, suggests that the inhibitory protein is a polyphenol oxidase. Research is being conducted to validate the presence of a soluble polyphenol oxidase in red clover extracts.

Table 1. pH and nitrogen fractions of red clover and alfalfa herbage and silage.

	Red Clover				Alfalfa			
	pH	FAA[a]-N	NH_3-N	NPN	pH	FAA-N	NH_3-N	NPN
		Greenhouse Study						
		Nitrogen (g kg^{-1} Total N)				Nitrogen (g kg^{-1} Total N)		
Inital herbage	6.48	39.5	3.2	135.4	6.17	59.9	3.8	160.8
	(0.011)[a]	(1.21)	(0.01)	(11.85)	(0.026)	(1.89)	(0.17)	(15.88)
Ensiled 7d	5.56	319.8	36.4	475.0	6.63	493.6	110.7	717.4
	(0.114)	(6.26)	(4.97)	(9.06)	(0.078)	(80.73)	(7.3)	(NA)[b]
		Field Study						
		Nitrogen (g kg^{-1} Total N)				Nitrogen (g kg^{-1} Total N)		
Initial herbage	6.12	35.1	3.5	113.5	6.30	47.2	6.7	144.5
Ensiled 7d	4.36	324.9	62.5	458.3	4.86	528.1	91.3	728.8
	(0.051)	(15.22)	(3.93)	(23.67)	(0.051)	(2.98)	(7.17)	(23.67)

[a]Standard deviations are in parenthesis, n =2.

[b]Value represents only one silo; no standard deviation calculated.

Characterization of Polyphenol Oxidase Isolated from Red Clover

B.A. Jones, R.D. Hatfield and R.E. Muck

Introduction

Red clover is known to undergo limited proteolysis in the silo and in a buffer extract. Interestingly this limited proteolysis is correlated to browning of the buffer extract. Browning in other plant systems is known to occur due to the activity of polyphenol oxidase (PPO), and prior research has demonstrated that red clover does contain a soluble PPO. Research was undertaken to isolate and characterize the soluble PPO present in red clover.

Material and Methods

Red clover leaves were harvested from greenhouse plants and immediately ground in liquid nitrogen. Degassed buffer (50 mM Tris, pH 7.0, 5 mM ascorbate) was added (1g leaves : 3 ml buffer) and the slurry was transferred to an anaerobic chamber and stirred for 15 min. Slurry was filtered and particulate material was removed by centrifugation in anaerobic tubes (20,000 x g, 25 min). Supernatant was collected anaerobically, acidified to pH 5.0 and centrifuged again. This supernatant was passed through an anion exchange column (QAE Sephadex) to remove ionized phenolics and the filtrate collected and loaded on a cation exchange column (TSK-Gel Toyopearl SP-650 M). Proteins were removed from the column by a linear gradient of NaCl (0 to 750 mM) and collected as individual fractions. Fractions with PPO activity were pooled. PPO activity was determined using 10 mM catechin (50 mM Tris, pH 7.0) as the substrate and absorbance changes were monitored at 435 nm or by running a native gel which was stained with 10 mM DOPA (50 mM Tris, pH 7.0).

Temperature and pH optima and pH stability were determined on the pooled fraction from the cation exchange column. PPO activity was monitored at temperatures between 10 and 60°C for 30 min, and the rate calculated over the linear portion of the curve. The pH optimum was determined using two substrates, catechin and DOPA+chlorogenic acid. Both substrates were used at 10 mM (30 mM tris:acetate buffer adjusted to the appropriate pH, pH range 3 to 9). Absorbance changes were determined at 435 nm for catechin and 485 nm for DOPA+chlorogenic acid. The pH stability was determined over the pH range 4 to 9. Equal portions of the PPO fraction and buffer (Tris:acetate adjusted to the correct pH) were mixed. Solutions were incubated for 0, 1, 2, 4, 24 and 48 h on ice and residual PPO activity determined using catechin (pH 7.0). Residual activity was expressed as a fraction of the activity in the initial extract.

Results and Discussion

The purification scheme increased the specific activity of PPO by 5.3 fold and removed the phenolic substrates that were involved in the browning reaction. The pH optima for PPO is depicted in Fig. 1 for both substrates. These

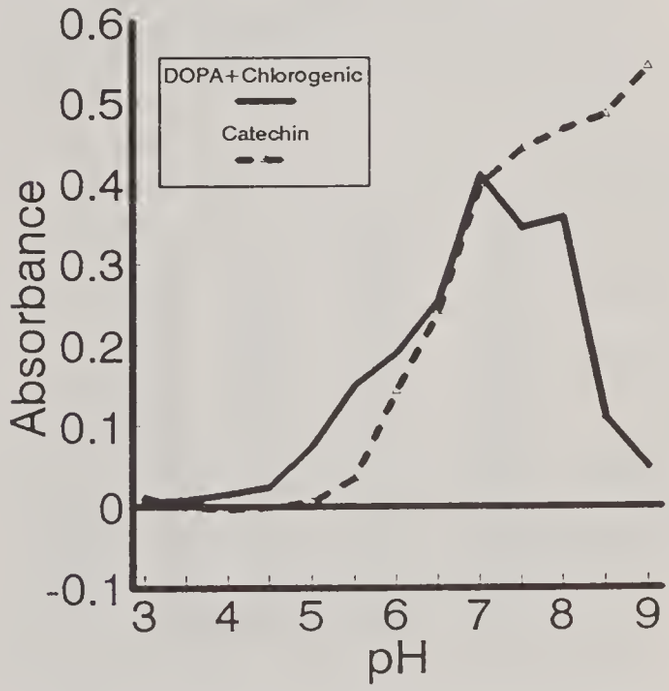


Figure 1. Effect of pH on polyphenol oxidase activity. Substrates were at 10 mM concentration in a 50 mM Tris:acetate buffer.

two substrates were selected because they are both diphenols, but catechin is a proanthocyanin phenol while DOPA and chlorogenic acid are phenolic acids. Both types of phenols are common in plants. With both substrates, PPO activity increased greatly from pH 5.5 to 7; however, substrate differences were observed at higher pHs. PPO activity using catechin continued to increase at high pH while that with DOPA+chlorogenic acid appeared to decline sharply above pH 8. This varied enzyme response to pH could be the result of pH effects on the substrate as well as the enzyme or both. Catechin has additional absorbance (not related to PPO activity) at the low pHs (3-5.5), but no absorbance due to pH at pH > 5.5. DOPA+chologenic acid had an opposite response and at pH >8.0 underwent auto-oxidation. Controls (boiled enzyme) were run at each pH for both substrates; however, due to the varied pH effect on the substrates, the accuracy of this correction will need to be checked. Regardless of the pH effects at high or low pH, the pH optima correspond to prior studies which note a broad pH optima over 7 to 8. The pH at which maximal stability of PPO was obtained was different from that of optimal activity. The greatest stability was noted at pH 6 (Fig. 2).

Optimum temperature for PPO activity was 30°C (Table 1). Increasing the temperature to 40 or 50°C resulted in lower activity (by 20%) but these activities were not significantly different ($p < 0.05$, T-test) from that at 30°C. PPO was denatured at 60°C. Interestingly, PPO retains 40% of its maximal activity at 10°C and corresponds to the observation that PPO is active (i.e., browning occurs in solutions) in the refrigerator unless inhibited.

Conclusions

A soluble PPO was partially purified from red clover leaves. The PPO had a broad pH optima (pH 7-8) regardless of the diphenol substrate used. Maximal stability of PPO was measured at pH 6.0. Maximal enzymatic activity was found at 30°C while 40% of this activity was still present at 10°C. Denaturation of PPO occurred at 60°C. Research will continue to purify PPO to homogeneity.

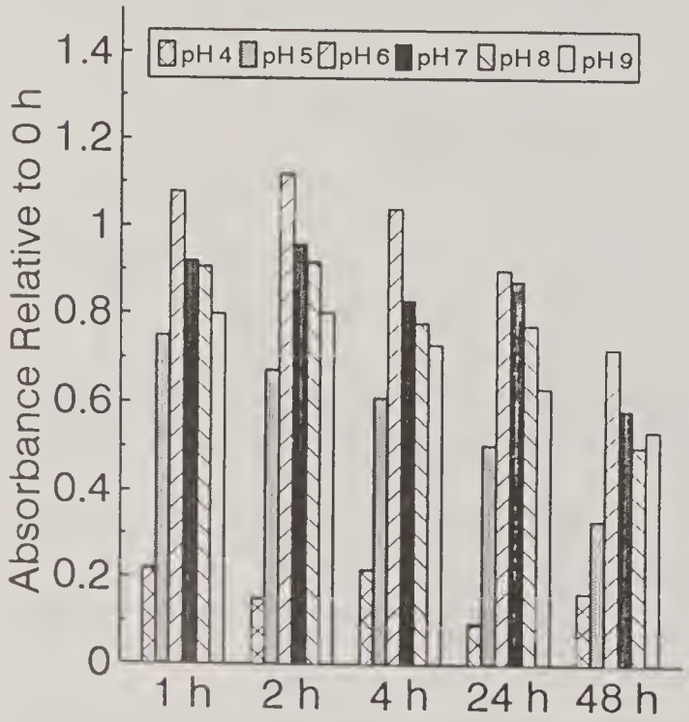


Figure 2. Stability of polyphenol oxidase at different pHs (30 mM Tris:acetate buffer).

Table 1. Effect of temperature on activity rates of polyphenol oxidase.

Temperature °C	Abs./mg protein.minute
10	.054 ± .007[1]
20	.067 ± .006
30	.141 ± .014
40	.112 ± .008
50	.115 ± .011
60	ND

[1]Means ± standard deviation, n = 2.

Correlation of Acid Detergent Lignin and Klason Lignin in Forages With *in Vitro* and *in Vivo* Dry Matter and Fiber Digestibility

H.G. Jung, D.R. Mertens and A.J. Payne

Introduction

Lignin content of forages has been shown repeatedly to be negatively correlated with forage fiber digestion. Among the most commonly accepted lignin assays is the acid detergent lignin (ADL) procedure of Van Soest utilizing sulfuric acid to remove cell-wall polysaccharides and leave a lignin residue after acid detergent extraction. Recently several members of the Cell Wall Group have demonstrated that the Klason lignin (KL) procedure, which also employs sulfuric acid for polysaccharide removal, provides a higher and possibly more accurate estimate of lignin content of forages, especially for grasses. A question still remained as to whether the Klason lignin assay gives a lignin determination which is correlated with forage fiber digestibility in the same manner as is ADL. We undertook a study to compare the ADL and KL procedures for their correlation with forage digestibility both *in vitro* and *in vivo*.

Materials and Methods

Twelve legume (alfalfa, red clover, Ladino clover and birdsfoot trefoil), 16 cool-season grass (barley, oat, smooth bromegrass, orchardgrass, tall fescue, and reed canarygrass), and eight warm-season grass (big bluestem, sorghum sudangrass, pearl millet, corn silage and corn stalklage) hays were analyzed for ADL and KL concentration. *In vitro* digestibility of dry matter (DM) and neutral detergent fiber (NDF) after 48 h of fermentation with mixed rumen microflora was determined on all hays. Nineteen of these 36 hays were also fed to sheep in a digestion trial where DM and NDF digestibilities were measured. The correlation between *in vitro* and *in vivo* digestibilities of DM and NDF and the ADL and KL estimates were determined.

Results and Discussion

As expected, the KL estimates of lignin content were greater ($P<0.05$) than the ADL values for these forage samples and the two methods were correlated ($r=0.75$. $P<0.05$). The relationships of digestibility to lignin method are shown in Figure 1. Across all forages, KL was negatively correlated ($r=-0.50$ to -0.82, $P<0.05$) with DM and NDF digestibility both *in vitro* and *in vivo*. The ADL estimate was also correlated ($r=-0.48$ to -0.80, $P<0.05$) with all digestibility measurements except for *in vivo* DM digestion ($r=0.22$, $P>0.05$). When the correlations for digestibility with lignin were compared within forage groups (legumes, cool-season, and warm-season grasses), it appeared that ADL was more frequently associated with digestibility than was KL (9/12 vs. 6/12 significant correlations for ADL and KL, respectively, Table 1).

Conclusion

It seems that the lignin fraction measured by the ADL method is more closely related to forage digestibility than KL among this set of hays. *In vitro* and *in vivo* digestibility correlations with lignin method were not always in agreement. The choice of lignin method

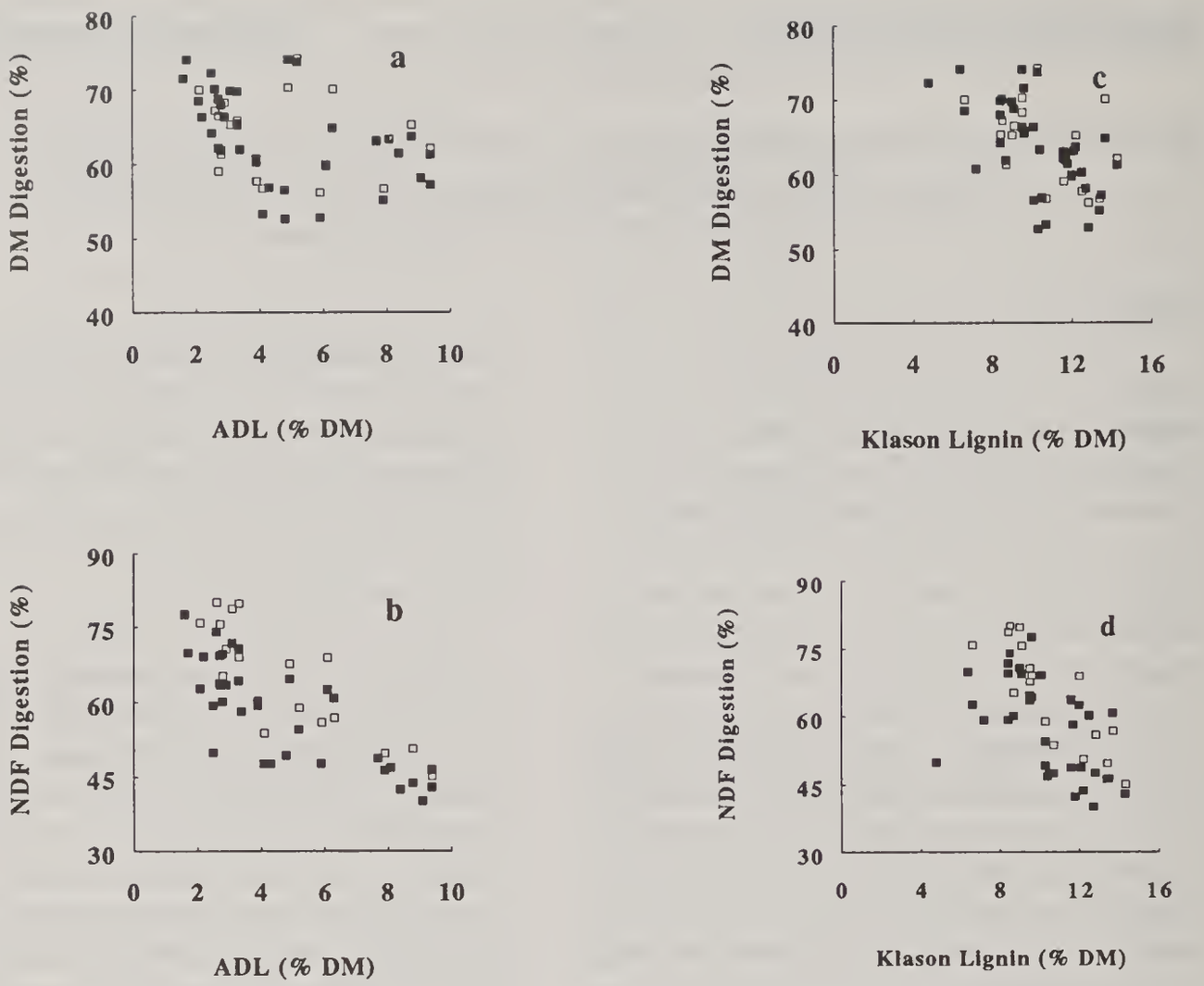


Figure 1. Digestibility of DM and NDF in vivo (□) *and* in vitro (■) *in relationship to ADL* (a & b) *and Klason lignin* (c & d) *content.*

will depend on the primary goal of the project. If prediction of digestibility of a forage sample is the primary goal, then the ADL procedure would seem to be the best choice. However, if accurate estimation of lignin content of forages is of equal or greater concern than prediction of digestibility, then the KL procedure may be the method of choice, especially for grasses where the difference in KL and ADL lignin estimates is a factor of two or three. Use of the KL procedure may reduce predictability of digestibility of forage samples, but whether this reduction is due to inaccuracy of the KL method or variability in the relationship of lignin with digestibility among different forage materials is not known.

Table 1. Correlation coefficients for lignin methods with digestibility.

	In Vivo		*In Vitro*	
	DM	NDF	DM	NDF
Legumes				
ADL	-.75*	-.90**	-.85**	-.89**
KL	-.63	-.75*	-.74**	-.42
Cool-season grasses				
ADL	-.64*	-.47	-.81**	-.67**
KL	-.83**	-.69**	-.74**	-.46*
Warm-season grasses				
ADL	-.96**	-.32	-.96**	-.59
KL	-.08	-.53	-.60	-.03

*,** P<0.10 and 0.05, respectively.

Variation in the Alfalfa Core Collection for Forage Quality Traits

H.G. Jung, C.C. Sheaffer and D.K. Barnes

Introduction

Alfalfa is the most widely grown perennial legume in American agriculture. Alfalfa is a very important forage crop for dairy production because of its relatively high protein content and low fiber levels. However, as production potential of dairy cows is improved, the nutritional quality of current alfalfa varieties is insufficient to meet the increased nutrient requirements of elite cows. Breeding for improved alfalfa forage quality has, therefore, become a major concern for alfalfa seed companies. Because the genetic base of elite alfalfa breeding lines is relatively narrow, interest has been expressed in broadening the germplasm used in breeding programs. To provide the alfalfa industry with information on the genetic diversity that exists in this species for forage quality traits, a trial was undertaken to assess forage quality of the alfalfa plant introductions in the US collection for this species.

Materials and Methods

Previous evaluation work on the US alfalfa collection of plant introductions (2300+) resulted in the identification of 226 entries, termed the CORE collection, that are believed to represent the range of genetic diversity in alfalfa. In 1991, 220 entries from the CORE collection, plus five contemporary check varieties, were planted at Rosemount, MN in a replicated field trial. Samples were collected from this nursery in 1992 after prior removal of the primary spring growth. Individual plots were harvested when the majority of the plants in the plot had reached flowering. Twenty-five flowering shoots were collected from each plot. Sampling was intended to equalize reproductive maturity among the entries, but not chronological age. All samples were lyophilized and sorted into leaf and stem fractions. Stems were scanned by NIRS and a 95 sample calibration set was analyzed for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and cellulose (CEL) concentration; *in vitro* ruminal 48 h digestibility of dry matter (IVDMD), and 24 h and 96 h digestibility of NDF (IVNDFD); and cellulose hydrolysis by a commercial fungal cellulase (CELD) for 24 and 96 h incubations. Quality of all alfalfa stem samples was estimated from the NIRS spectral calibrations.

Results and Discussion

Because of winter injury, only 215 of the CORE collection entries, plus the five check varieties, were available for study. As can be seen in Table 1, variation among entries was found for all forage quality traits. Range of CP concentration and CELD after 96 h of incubation were the traits with the largest variability in this study. The rest of the digestibility measures were also relatively variable and, interestingly, ADL was of a similar degree of variability among the entries. The concentrations of NDF, ADF and CEL were the least variable of the forage quality traits. A high degree of correlation was found among the forage quality traits (Table 2). As expected, ADL was negatively correlated with all measures of digestibility. There was also significant positive correlation among digestibility traits, including the enzymatic assay and the *in vitro* ruminal digestibilities.

Conclusion

The data clearly indicate that the US alfalfa germplasm collection contains substantial variation for many forage quality traits of

interest to industry. A similar evaluation of diversity of forage quality in current commercial varieties and breeding materials is underway. In combination, these data will help industry decide if it will be necessary to include the plant introduction germplasm in future development of improved alfalfa varieties.

Table 1. Variation in chemical composition of stems among alfalfa CORE collection entries and five contemporary check varieties for forage quality traits.*

Trait	Mean	Standard deviation	Minimum	Maximum
		g kg^{-1} DM		
CP	92	6	74	115
NDF	658	18	602	693
ADF	564	15	505	590
ADL	119	5	99	131
CEL	404	13	362	430
		g kg^{-1}		
IVDMD	465	20	432	548
NDFD				
24 h	239	12	198	271
96 h	335	14	300	388
CELD				
24 h	297	18	257	355
96 h	336	23	291	468

*All traits exhibited significant ($P<0.01$) variation among entries.

Table 2. Correlations among the forage quality traits for the alfalfa CORE collection entries and the five contemporary check varieties.

	CP	NDF	ADF	ADL	CEL	IVDMD	NDFD 24 h	NDFD 96 h	CELD 24 h	CELD 96 h
CP	—									
NDF	-.71	—								
ADF	-.67	.96	—							
ADL	-.48	.37	.42	—						
CEL	-.54	.90	.91	.18	—					
IVDMD	.68	-.68	-.75	-.64	-.50	—				
NDFD 24 h	.38	.14	NS[a]	-.44.	36	.47	—			
NDFD 96 h	.42	-.34	-.42	-.89	-.14	.71	.46	—		
CELD 24 h	.38	-.33	-.32	-.73	NS	.60	.52	.71	—	
CELD 96 h	.29	-.32	-.43	-.61	NS	.80	.52	.74	.63	—

[a]Non-significant ($P>.05$).

Harvest Management Effects on Red Clover Forage Yield, Quality, and Persistence

R.R. Smith, D.W. Wiersma, M.J. Mlynarek, R.E. Rand, D.K. Sharpee and D.J. Undersander

Introduction

Cultivars of red clover (*Trifolium pratense* L.) that have been developed during the last two decades are more persistent than those developed prior to the 1970's. The current practice in the upper midwest region of the U.S. of harvesting forage legume crops early (bud stage of growth) and more frequently (three harvests prior to 1 Sept.) to obtain high quality forage may negate improved persistence and shorten stand life of red clover. Therefore, the objective of this study was to evaluate forage yield, quality, and persistence of five red clover cultivars harvested at different frequencies throughout the growing season.

Materials And Methods

In 1989 and 1990, five red clover cultivars developed in the 1970's and 1980's (Atlas, Arlington, Marathon, Reddy, and Red Star) were established on the University of Wisconsin-Madison Agricultural Research Stations at Arlington, Ashland, Marshfield, and Spooner, WI. Five harvest management regimes (Table 1) were imposed on all cultivars at each location.

Harvest management regimes were whole plots, and cultivars were sub plots in a split plot arrangement of treatments in a randomized complete block design with four replications. Due to severe winter injury at Arlington, Ashland, and Spooner in the winter of 1991-92, only data measured on plots established in 1989 are presented. All plots were harvested according to the management regimes (Table 1) in 1990 and 1991 and at the bud stage of growth in 1992 (fourth year). The single fourth year harvest was used to assess forage yield potential after three years of production under various management regimes.

Forage yield (tons dry matter per acre) was determined using a small plot forage harvester. Crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) concentrations (g kg^{-1}) were determined from samples of each replicate of the cultivar Arlington at each harvest using Near Infrared Reflectance Spectrometry (NIRS) technology.

Results

Cultivar differences were small and consistent between management regimes and locations and will not be discussed further. Significant management regime by location and year interactions were observed, but the differences were due to magnitude and not direction; therefore, data averaged over locations will be presented.

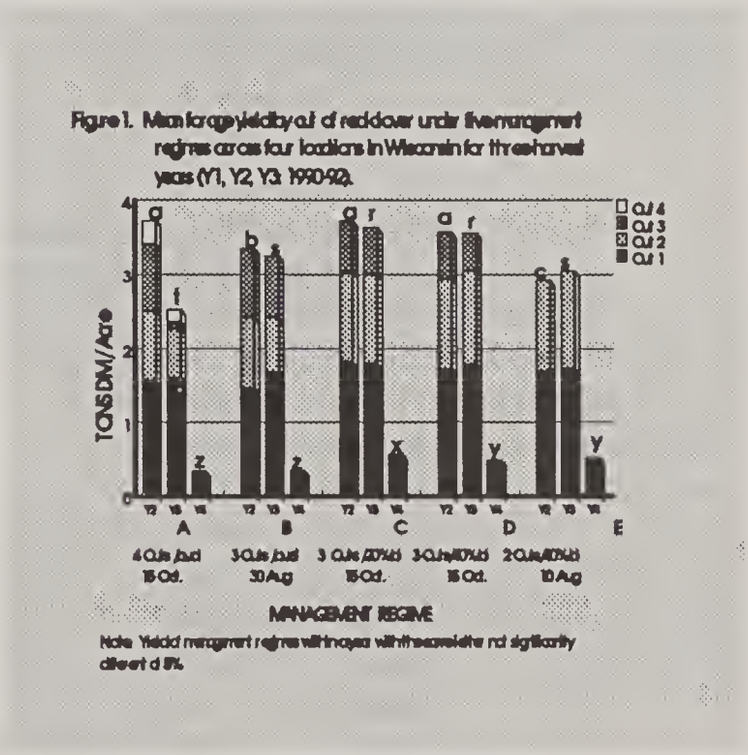


Figure 1. Mean forage yield by cut of red clover under five management regimes across four locations in Wisconsin for three harvest years (Y1, Y2, Y3: 1990-92).

Significant differences were observed between harvest management regimes for total forage yield each year (Fig. 1). Harvesting red clover three times, twice at the 20% bloom stage before 1 Sept. and again in the late fall (15 Oct.) (Management Regime C), produced the most total forage over the two year period. Frequent and early harvest (Management Regime A) produced the greatest amount of forage in the second year, but forage production was reduced in the second and subsequent harvests of the third year relative to the other Management Regimes. Harvesting the forage twice in the second year before 1 Sept. at either 20 or 40% bloom (Management Regimes C, D, and E) did not reduce forage yield in the subsequent year. In addition, taking a late fall (15 Oct.) harvest in the second year did not affect forage yield in the third year for these Management Regimes (C and D). Harvesting frequently and at the early stages of growth (bud) reduced subsequent yield each year after the second year.

Crude protein concentration of the red clover forage declined and ADF and NDF concentration increased as the forage advanced in maturity. At the 20 and 40% bloom stage of growth, CP concentration was slightly below a desirable level for both of the first two harvests and ADF concentration was higher than desirable in the first harvest. NDF concentration only exceeded 400 g kg^{-1} at the 40% bloom stage in the first harvest.

Harvesting frequently at early stage of growth (Management Regimes A and B) produced the least amount of forage in the spring of the fourth year (Fig. 1). This practice reduced the persistence of all red clover cultivars and was quite evident in the late summer and early fall of the third year.

Conclusions

Total two-year forage production was greatest when red clover cultivars were harvested three times (two cuts before 1 Sept. and 15 Oct.) beginning at either the 20 or 40% bloom stage of growth. Harvesting red clover early (bud stage of growth) and frequently (three times prior to 1 Sept.) produced less total forage of high quality and reduced persistence of the forage stands. Forage quality as measured by CP, ADF, and NDF concentration declined with increasing plant maturity, primarily in the first cut, but was in a range of acceptability. To retain longevity of red clover stands in the upper midwest U.S. (above the 43 N latitude), it would appear that it is not desirable to harvest the forage more than twice prior to 1 Sept.

Table 1. Red clover harvest management regime, growth stage at harvest, number of cuts and average cutting date.

Man.	Growth Stage	No. of Cuts	Average Cut Date
A	Bud	4	10 Jun, 10 Jul, 30 Aug, 15 Oct
B	Bud	3	10 Jun, 10 Jul, 30 Aug
C	20%	3	20 Jun, 30 Jul, 15 Oct
D	40%	3	25 Jun, 10 Aug, 15 Oct
E	40%	2	25 Jun, 10 Aug

Unreduced Gametes in Ball Clover and Its Relevance in White Clover Breeding

S. Bullitta, R.R. Smith, G.M. Scarpa and F. Veronesi

Introduction

The importance of white clover (*Trifolium repens* L. 2n=4x=32) as a forage legume, fixer of atmospheric nitrogen and cover crop is widely recognized. Ball clover (*Trifolium nigresces* Viv. 2n=2x=16), a diploid species naturally occurring in the pastures of the Mediterranean region, is indicated as a wild ancestor of white clover, a tetraploid. Ball clover could be a valuable source of germplasm for incorporating desirable traits into white clover such as tolerance to low soil fertility, increased seed production, winter hardiness, etc. The objectives of this research were to ascertain the presence of 2n gametes (unreduced gametes) in ball clover and, if such exists, to use them to obtain 4x hybrids between ball clover (2x) and white clover (4x).

Materials and Methods

Ball clover plants used in the experiment were selected from 20 natural diploid populations collected on the Island of Sardinia, Italy. Four to 18 plants from each population were examined. Two-hundred-fifty pollen grains were examined morphologically for each of three flowers of each plant. Ploidy level of the pollen grain was based on the morphology of each grain when dry. Haploid (n) pollen grains in ball clover are oblong and regular in shape and diploid (2n) pollen grains are triangular, square or irregular.

Results

Unreduced gametes (2n pollen) were detected in 17 of the 20 populations examined (Table 1). Although the frequency of 2n pollen in some populations and some plants was quite low, other populations, such as #34, had 67% of the plants producing 2n pollen. Populations #12 and #37 each had a plant which produced over 30% 2n pollen.

Self incompatible white clover plants were used as female parents and crossed to high 2n pollen producing ball clover plants. Individual white clover plants were placed in isolation cages with the ball clover plants and intercrossed using honeybees. The 13 plants obtained from these crosses were characterized by a low level of pollen stainability (4.3%). The interspecific origin of the 13 plants was confirmed by root tip chromosome counts and all the plants were triploid (2n=3x=24). In addition, the plants were intermediate to the parental species for central leaflet length and width.

From the practical point of view, the absence of tetraploid interspecific hybrids presents a problem in the attempts to utilize ball clover directly as a source of desirable traits. However, subsequent backcrossing of the triploids to white clover may recover some of the ball clover characteristics. Selection in ball clover for increased frequency of 2n gametes may also aid in the production of tetraploid interspecific hybrids.

Conclusions

The fact that 2n gametes are produced in ball clover and that hybrids are produced between white and ball clover would suggest that further investigations are warranted to elucidate the relationship between white clover and its progenitors. In addition, further research is needed to enhance the introgression of ball clover germplasm into white clover utilizing the 2n gametes functional in ball clover.

Table 1. Number of plants producing 2n pollen and distribution of production in twenty Italian natural populations of ball clover.

Population	Plants evaluated	Plants producing 2n pollen	Range of 2n pollen in the 2n pollen producers
10	10	6	*
11	4	1	*
12	11	4	1.3 - 34.0
14	17	7	1.3 - 2.6
18	15	2	*
20	12	2	1.3 - 2.6
22	18	8	1.3 - 3.4
23	8	2	1.3 - 2.6
24	7	/	/
25	5	/	/
29	11	2	4.0 - 5.3
30	4	/	/
33	17	6	1.3 - 17.3
34	15	10	1.3 - 10.5
36	11	5	1.3 - 32.0
238	10	6	1.3 - 13.3
239	14	8	1.3 - 13.3
240	14	7	1.3 - 10.6
241	8	4	1.3 - 2.6

Release of Regenerative Red Clover Germplasm, NEWRC

R. R. Smith and K.H. Quesenberry

Introduction

NEWRC red clover (*Trifolium pratense* L.) germplasm was developed and released jointly by the USDA-ARS, Florida Agricultural Experiment Station and Wisconsin Agricultural Experiment Station. This germplasm has high plant regeneration from callus tissue culture (72%) and has use in genetic transformation and breeding research.

Breeding Procedures and Selection Methods

NEWRC was developed by applying five cycles of recurrent phenotypic selection for increased plant regeneration via somatic embryogenesis from callus tissue from the cultivar Arlington. A three step tissue culture protocol (callus induction, embryo induction, and plant development) based on Gamborg's B5 basal salts, NAA and 2,4-D as auxins, and kinetin and adenine as cytokinins was utilized. Hypocotyl tissue was used as the explant source in cycles 1 and 2 and petiole tissue was used for cycles 3, 4, and 5. Plants in each cycle were selected on the basis of plantlet regeneration frequency in culture. Only original explant source plants were used in each cycle for intercrossing. This was done to eliminate any possible negative somaclonal variation which might have been introduced in

the culture phase. The number of plants selected and intercrossed in each generation and the regeneration frequency are reported in Table 1. The percentage of NEWRC plants capable of regeneration is 72 in contrast to the original population, Arlington, with a 4% regeneration percentage. In the developmental phase the germplasm was designated RSP5

Conclusions

Released germplasm is in the first generation of synthesis (Syn 1). Because the original population was the cultivar Arlington, the germplasm should have good adaptation and a relatively good resistance to *Kabatiella caulivora* (Kirchn.) Karak., causal agent of the disease northern anthracnose.

Small quantities (up to 10 grams) of seed of NEWRC are available upon written request to K.H. Quesenberry, Department of Agronomy, University of Florida, Gainesville, FL 32611 or R.R. Smith, USDA-ARS, US Dairy Forage Research Center, 1925 Linden Drive West, Madison, WI 53706.

Table 1. Number of plants evaluated and selected for intercrossing and percentage regeneration for the base population and five cycles of selection.

Population	Cycle	Plants evaluated	Plants intercrossed	Percent regeneration
		no.	no.	%
Base (Arlington)	C0	200	8	4
RPS1	C1	100	8	8
RPS2	C2	80	13	16
RPS3	C3	140	26	20
RPS4	C4	170	51	38
RPS5 (NEWRC)	C5	50	—	72

A New Hypothesis on the Role of Cell Wall Accessibility and Structure on Digestion of Forages

J.R. Wilson and D.R. Mertens

Introduction

Digestion and intake of forages is usually limited by characteristics of the plant cell wall. The cell wall of forage is slowly and incompletely digested and must be degraded by microbial enzymes. Research on cell wall digestion has focused primarily on the limitations imposed by the chemical nature of cell walls. In most studies, the role of lignification, phenolic acid cross-linking, and three-dimensional structure of the major carbohydrates in cell walls (cellulose and hemicellulose) has been emphasized. Our objective was to propose and evaluate an alternative hypothesis about the limitations to cell wall digestion - that the anatomy of plants and the architecture of their tissues and cells may be significant barriers to digestion.

Methods

Data on the dimensions of microbes and plant tissues and cells were obtained from our research and published literature. Rate of digestion per unit of surface area was derived

from the work of Chesson et al. (1986. J. App. Bact. 60:327-336). Rate of penetration of bacteria into the lumen of long fiber cells was estimated assuming diffusion rather than capillary action was the main mechanism of migration of bacterial cells. The effects of physical accessibility, particle size, and surface area on digestion of plant cells were calculated based on physical laws and estimated rates to demonstrate the consequences of the new hypothesis.

Results and Discussion

Enzymes needed to hydrolyze the complex polysaccharides in forage cell walls (cellulose and hemicellulose) are typically bound to the cell walls of ruminal bacteria. This arrangement of the enzymatic complex requires that the microbe be in very close proximity (if not attached) to the cell wall substrate for digestion to occur. It insures that the sugars that are released during hydrolysis of complex polysaccharides are absorbed rapidly by the microorganism doing the digestive work. However, the requirement that microbes be attached or in close proximity to cell walls when digesting them illustrates the potential limitation that might occur if plant tissues or cells are not readily accessible to bacteria.

Analysis of microbial digestion of plant cell walls and the physical dimensions and rates of the process reveals the potential for accessibility to limit digestion of forages. The following factors indicate the role that accessibility may play in digestion which may be independent of the role of chemical and physical barriers at the molecular level:

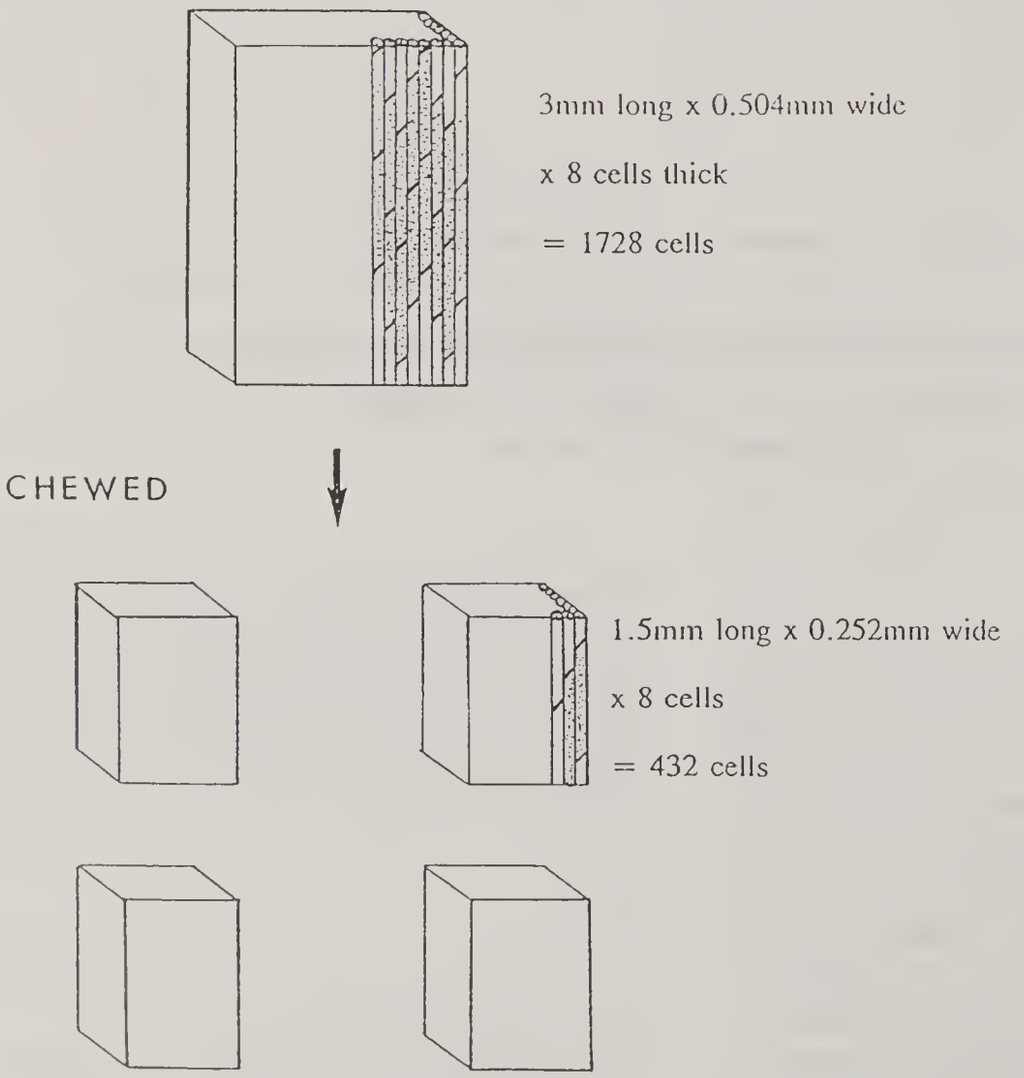


Figure 1. Diagram showing the potential number of non-disrupted cells that would be difficult for bacteria to penetrate and digest for a particle of sclerenchyma cells (upper section). The lower section illustrates the anticipated particle size after chewing has occurred. This size particle would also be eligible for escape from the rumen.

(1) Digestion is confined to the lumenal surface of cell walls because the middle lamella/primary cell wall regions of cells are relatively indigestible. Thus, bacteria must gain access to the interior of plant cells before significant cell wall digestion can occur. Even with effective chewing by ruminants, individual particles of plant tissues contain hundreds of cells, few of which will be ruptured to allow rapid bacterial access (Fig. 1).

(2) Many plant cells are long and tubular in structure. After bacteria gain access to the lumen of one end of a 1 mm long sclerenchyma cell, we estimate it would take 17 days to progress to the distal end of the plant cell based on diffusion and digestion rates. Under these conditions, digestion of these cell types would be limited by the time particles remain in the rumen (Fig. 2).

(3) The secondary cell walls of plants are thick in relation to rates of digestion. We calculate that at best only 0.45 to 0.60 µm of wall thickness (as little as 20% of the total cell wall weight) would be digested within the average residence time of fiber particles in the rumen.

(4) Digestion of cell walls is surface-based and many cells have a low ratio of surface area (SA) to volume of cell walls (CWV). Calculated SA:CWV ratios for single cells are: 0.2:1 for sclerenchyma, 1.9:1 for parenchyma, and 6.7 to 13.3:1 for mesophyll. Differences in SA:CWV ratio may partially explain the differences in the extent of digestion for these cell types that are typically observed, i.e., mesophyll>parenchyma>sclerenchyma.

Conclusion

A testable hypothesis that anatomical and architectural characteristics of plant tissues and cells may be a significant impediment to digestion of forage cell walls has been developed. This hypothesis is supported by available observations. More research is needed to evaluate the hypothesis that physical accessibility of plant cell walls to microbial attachment or association is a major factor limiting digestion and to determine the relative importance of chemical and accessibility factors affecting digestion.

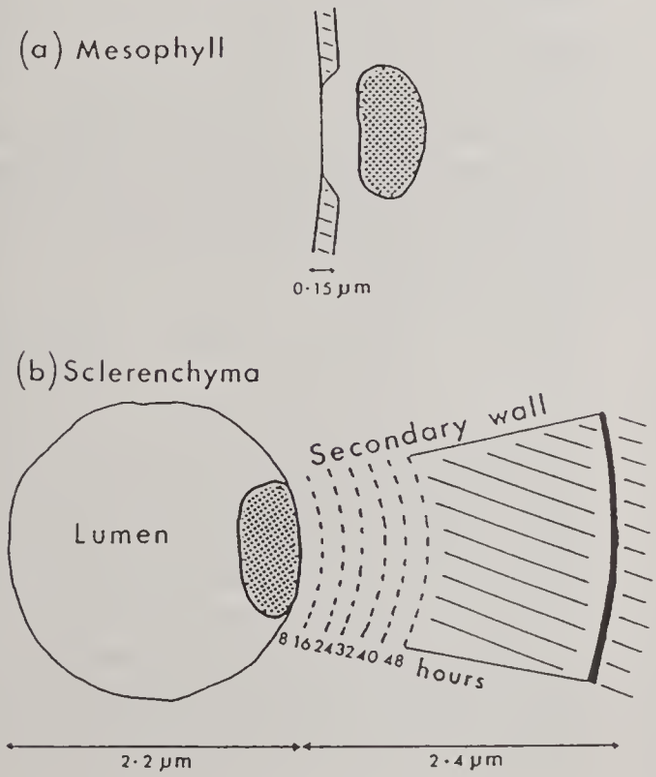


Figure 2. Illustration of a bacterium digesting a mesophyll cell (a) or sclerenchyma cell (b) drawn to scale showing the progressive fronts of digestion over 8 to 48 hours based on a digestion rate of 0.15 mm/8hr.

Endogenous Metabolism of *Fibrobacter Succinogenes* and Its Relationship to Transport, Viability and Cellulose Digestion

J.E. Wells and J.B. Russell

Introduction

Mammals are unable to digest cellulose, but ruminants have bacteria which degrade this material. Although ruminal bacteria are efficient digesters of cellulose, the rate of digestion is often slow and *in vitro* experiments indicated that there can be a pronounced lag before the initiation of degradation. The lag period has usually been attributed to bacterial attachment to cellulose (Van Soest 1982). Many ruminal bacteria die rapidly (Mink and Hespell 1981a; Mink and Hespell 1981b; Leedle *et al.* 1982; Wachenheim and Hespell 1985), but the relationship between viability and lag time of fermentation has not been considered.

Halliwell and Bryant (1963) compared various strains of ruminal cellulolytic bacteria and noted that *F. succinogenes* S85 was the most active digester of crystalline cellulose. Costerton *et al.*, (1974) noted that *F. succinogenes* accumulated large amounts of glycogen (Stewart *et al.*, 1981), but early work by Bryant *et al.* (1956) indicated that *F. succinogenes* was only viable for short periods of time. The following experiments were designed to: 1) examine why *F. succinogenes* died so quickly, and 2) correlate its rate of endogenous metabolism with its ability to digest cellulose.

Materials and Methods

F. succinogenes was grown anaerobically in the medium of Caldwell and Byrant (1966) and either cellobiose or cellulose was used as an energy source. Viability was determined by the most probable number method using 10 fold dilutions. ATP was determined by the firefly assay using luminescence as a detection method. Intracellular potassium from cells which were isolated through silicon oil was determined by flame photometry. Cellobiose transport was estimated from the uptake of tritium labeled cellobiose. Cellular polysaccharide was estimated by the anthrone method and cellular protein was estimated by the method of Lowry *et al.* (1951). Membrane potential ($\Delta\Psi$) was estimated from the uptake of radiolabeled tetraphenylphosphonium. Cell attachment was estimated from the turbidimetric assay of Minato and Suto (1976).

Results and Discussion

Fibrobacter succinogenes S85 digested ball-milled cellulose at a rapid rate (0.10 h^{-1}), but there was a long lag time if the culture was not transferred daily. When *F. succinogenes* was starved for 100 h, a large fraction of the cells (>30%) still bound to cellulose, but the lag time was 150 h. The lag time was similar for either cellulose- or cellobiose-grown inocula, and lag times were highly correlated ($r^2 = 0.91$) with a decrease in viable cell number. The number of viable cells declined from 108 to 106 in the first 30 h of starvation, and by 72 h the viable cell number was less than 103/ml. Cells growing exponentially on cellobiose had a large pool of polysaccharide, and continuous culture experiments indicated that polysaccharide accumulation was not significantly influenced by the growth rate of the culture (approximately 0.7 mg polysaccharide/mg protein). When the

cellobiose was depleted, cellular polysaccharide decreased at a first order rate of 0.09 h^{-1}. The rate of endogenous metabolism was initially 0.08 mg polysaccharide/mg protein/h, and there was little decline in viability until the rate of endogenous metabolism was less than 0.01 mg polysaccharide/mg protein/h. When the rate was less than 0.01 mg polysaccharide/mg protein/h, cells could not maintain a sodium gradient, transport cellobiose or grow.

Escherichia coli carefully regulates its rates of glycogen synthesis and glycogenolysis (Preiss 1984; Senez and Belaich 1965), but *F. succinogenes* cannot regulate this aspect of its metabolism. Goutet *et al.* (1992) showed that exponentially growing *F. succinogenes* continuously synthesized and degraded glycogen. Our work indicated that the polysaccharide content of stationary cells decreased in a first order fashion (9% per h).

The terms "maintenance energy" and "endogenous metabolism" have often been used interchangeably (Dawes 1985), but the maintenance rate of *F. succinogenes* was at least 2-fold greater than the initial rate of endogenous metabolism and 20-fold greater than the minimum endogenous rate needed for cell viability. Based on this comparison, the maintenance rate and the endogenous rate are not synonymous.

Because the rumen usually contains an abundance of solid feed material, one might argue cellulolytic bacteria like *F. succinogenes* would not be subjected to significant periods of energy starvation. This argument, however, ignores the fact that the fresh feed must be colonized and the observation that cellulose is often surrounded by hemicellulose (Begin, 1992). *F. succinogenes* can degrade hemicellulose, but it is unable to utilize the degradation products as an energy source (Dehority, 1973).

Conclusions

The ruminal bacterium *F. succinogenes* dies quickly because it is unable to regulate its rate of endogenous metabolism. The rapid depletion of the energy reserves in the early phases of starvation lead to a premature decrease in ATP, $\Delta\Psi$, pNa, transport activity, and viability. Rapid declines in viability cause significant increases in the lag time of cellulose digestion.

The Ability of *Acidaminococcus Fermentans* to Oxidize Trans-aconitate and Prevent the Accumulation of Tricarballylate, a Toxic End-product of Ruminal Fermentation

G.M. Cook, J.E. Wells and J.B. Russell

Introduction

Grass tetany, a potentially fatal disease of mature ruminants, has been recognized since the 1930's, but the etiology of the hypomagnesemia is perplexing. In the 1960's, Burau and Stout found a strong correlation between the trans-aconitic acid content of grasses and the incidence of this disease. Based on capacity of trans-aconitate to chelate magnesium, it appeared that trans-aconitate was decreasing magnesium absorption. This hypothesis was supported by the demonstration that oral doses of trans-aconitic could decrease blood magnesium. The direct involvement of trans-aconitic acid in grass tetany was, however, contradicted by the observation that trans-aconitate had a short half life in ruminal fluid. Subsequent work showed that ruminal bacteria rapidly converted trans-aconitate to tricarballylate, another tricarboxylic acid. Sheep given trans-aconitic acid and

cattle fed grasses containing trans-aconitic acid absorbed tricarballylic acid. Rat studies showed that tricarballylic acid, by chelating blood magnesium, could increase the excretion of magnesium and other divalent cations. Trans-aconitate was stoichiometrically converted to tricarballylic acid by *Selenomonas ruminantium*, a common bacterium in grass-fed ruminants. Mixed ruminal bacteria also converted some of the trans-aconitate to acetate, a normal and non-toxic end-product of ruminal fermentation, but the organisms responsible for this conversion were not initially isolated. Recently we showed that trans-aconitate was converted to acetate, carbon dioxide and hydrogen by *Acidaminococcus fermentans*.

Materials and Methods

Acidaminococcus fermentans and *Selenomonas ruminantium* were grown anaerobically in the medium of Caldwell and Bryant. Trans-aconitate, citrate or glucose was added after autoclaving. Fermentation end-products were analyzed by high pressure liquid chromatography. *A. fermentans* was added to mixed ruminal bacteria from the rumen of a cow, and the conversion of trans-aconitic acid to tricarballylate was monitored *in vitro*.

Results and Discussion

When *S. ruminantium* and *A. fermentans* were co-cultured with trans-aconitate and glucose, tricarballylate never accumulated and all the trans-aconitate was converted to acetate. Continuous culture studies (dilution rate of 0.1 h^{-1}) likewise indicated that *A. fermentans* could out-compete *S. ruminantium* for trans-aconitate. Mixed ruminal bacteria converted 45% of the trans-aconitate to tricarballylate, but there was a 50% decrease in tricarballylate production if even small amounts of *A. fermentans* were added (0.01 mg protein/mg mixed bacterial protein). When *A. fermentans* (2 g bacterial protein) was added directly to the rumen, the subsequent conversion of trans-aconitate to tricarballylate decreased 50%, but this effect did not persist for more than 18 h. The eventual increase in tricarballylate production, however, indicated *A. fermentans* did not persist in the rumen.

The dilution of *A. fermentans* from the rumen is probably related to its relatively narrow range of potential substrates. The grass hay which was fed to the cow did not contain significant amounts of trans-aconitate, citrate or pyruvate. The hay did contain the amino acid glutamate, another potential energy source for *A. fermentans*, but this amino acid is rapidly deaminated by a mixed ruminal bacteria. Trans-aconitate accumulation in plants is a transient and somewhat unpredictable event only observed in grazing situations, and commercial trans-aconitate is expensive. Consequently, it was not possible to feed the cow a diet which contained trans-aconitate.

The inoculation of natural habitats with bacteria has usually been confounded by the ubiquitous nature of bacteria and the dependence of all bacteria for a suitable niche. These general principles of microbial ecology, however, do not account for sudden and transient changes in the substrate availability. Grasses accumulate trans-aconitate very rapidly, and there is often little if any trans-aconitate prior to the accumulation. Because *A. fermentans* numbers in the rumen are normally only 10^2/ml (6), and mixed ruminal bacteria are greater than 10^{10} ml, *A. fermentans* cannot initially compete with other bacteria for the trans-aconitate. By the time *A. fermentans* is able to grow up to a significant density, the cow has already been exposed to a lethal accumulation of tricarballylate.

Conclusions

Further work is needed to see if ruminal inoculation of *A. fermentans* can provide a means of decreasing tricarballylate production and decreasing mortality.

The Energy Spilling Reactions of *Streptococcus Bovis* and the Resistance of Its Membrane to Proton Conductance

G.M. Cook and J.B. Russell

Introduction

In ruminant animals, microbial protein is the major source of amino acids, and the flow of microbial protein from the rumen can have a dramatic effect on animal performance. The growth yields of ruminal bacteria are generally high, but more than 1/3 of the total ATP production can be devoted to non-growth energy expenditures. This non-growth energy has typically been described as maintenance energy, but preliminary experiments indicated that non-growing mixed ruminal bacteria could consume glucose faster than the maintenance rate.

Recent work with the ruminal bacterium, *Streptococcus bovis*, indicated that non-growth energy dissipation could be completely eliminated by N,N-dicyclohexylcarbodiimide (DCCD), an inhibitor of the F_1F_0 ATPase. Based on this result, it appeared that a futile cycle of protons through the cell membrane was causing a turnover of ATP. Because glucose-limited cells did not spill energy, it appeared that the energy spilling mechanism might be regulated. The aims of the following experiments were to: 1) determine proton flux through the cell membrane (amperage), 2) compare proton flux with the rate of ATP turnover by energy spilling cells, and 3) correlate membrane resistance changes with the rate of proton flux and energy spilling.

Materials and Methods

Streptococcus bovis JB1 was grown anaerobically at 39°C in basal medium containing salts, cysteine hydrochloride, Trypticase, yeast extract and glucose. Bacterial heat production was measured with an LKB model 2277 Bioactivity monitor. The glucose consumption rate was estimated from the enthalpy (ΔH) of glucose conversion to lactate (21 cal or 88.2 j/mmol glucose), and the conversion factor, 1.16 mW/cal/h. The electrochemical proton gradient (Δp) was estimated from the uptake of radiolabeled TPP^+, benzoate, PEG, or H_2O. Adenine nucleotides were extracted from the cells by perchloric acid /EDTA treatment after separation of the cells from the growth medium. Adenine nucleotides were determined by the luciferine-luciferase method. The determination of intracellular phosphate concentration was carried out in cell extracts which were prepared according to the method of Bulthuis *et al.* Glucose was analyzed by an enzymatic method using hexokinase and glucose-6-phosphate dehydrogenase. Protein from NaOH-hydrolyzed cells (0.2 M NaOH, 100°C, 15 min) was assayed by the method of Lowry *et al.* Cellular polysaccharide was assayed by the anthrone method. Lactate, ethanol and fermentation acids in cell-free supernatant samples were analyzed by high pressure liquid chromatography.

Results and Discussion

Glucose-excess cultures of *Streptococcus bovis* consumed glucose faster than the amount that could be explained by growth or maintenance, and non-growing chloramphenicol-treated cells had a rate of glucose consumption that was 8-fold greater than the maintenance rate. Because the energy spilling could be eliminated by N,N-dicyclohexylcarboiimide (DCCD), an inhibitor of the membrane-bound F_1F_0 ATPase, and increased by 3,3',4',5-tetrachlorosalicylanilide (TCS), a protonophore, it appeared that a futile cycle of protons through the cell membrane was responsible for most of the energy spilling. When the rate of energy spilling was decreased gradually with iodoacetate, there was only a small decrease in the phosphoryla-

tion potential ($\Delta G'p$) and the H^+/ATP decreased from 4.2 to 3.6. Based on the $\Delta G'p$ estimate of H^+/ATP and the rate of ATP production, the flux of protons (amperage) across the cell membrane increased with the rate of energy spilling. Amperage values estimated from $\Delta G'p$ were, however, nearly twice as great as the values which were estimated from the heat production (ΔH) of the cells (amperage = 0.38 x wattage ÷ Δp). This latter comparison indicated that only a fraction of the ΔG of ATP hydrolysis was harvested by the F1F0 ATPase to pump protons. Both estimates of amperage indicated that the resistance of the cell membrane to proton conductance was inversely proportional to the log of the energy spilling rate.

Conclusions

Based on these results, it appears that *S. bovis* will spill energy whenever there is an excess of energy and the catabolic rate is inhibited by the availability of other nutrients or its capacity to synthesize amino acids. If *S. bovis* were the only bacterium in the rumen and its growth was completely inhibited, the rate of energy spilling could be as great as 3 kg glucose per h. Experiments are currently under way to estimate the rate of energy spilling by mixed ruminal bacteria.

Phosphoenolpyruvate Carboxykinase from *Ruminococcus flavefaciens* FD-1

L. Schöcke and P.J. Weimer

Introduction

The reversible carboxylation of phophoenolpyruvate (PEP) to oxaloacetate (OAA), catalyzed by PEP carboxykinase (PEPCK), is the first committed step in the production of succinate by ruminal cellulolytic bacteria. Thus, the regulation of activity of this enzyme is potentially important in the partitioning of fermentation endproducts into propionate versus acetate within the rumen. This enzyme has been studied primarily in *Escherichia coli* and in several higher organisms. There are no reports describing this enzyme in any strict anaerobe or ruminal microbe.

Methods

R. flavefaciens was grown at 39°C under CO_2 in 12 L carboys that contained a modified Dehority's medium lacking trypticase and rumen fluid and containing 4.5 g cellobiose / L as fermentable carbohydrate. Cells were concentrated using tangential-flow filtration and pelleted by centrifugation. The resulting cell paste was resuspended in 5 mM phosphate buffer (pH 8.0) that contained 2 mM $MgCl_2$ and 0.02 mM $MnCl_2$ and passed through a French pressure cell at 18,000 psi. The broken cell suspension was collected under CO_2, centrifuged at 35,300 x g, and the resulting supernatant recovered as the crude extract.

The enzyme was purified by a four-step procedure involving: 1) ammonium sulfate precipitation; 2) desalting with BioGel P6; 3) two-stage isoelectric focusing with a Bio-Rad Rotofor system using 3/10 ampholytes; and 4) chromatographic buffer exchange with BioGel P6 and 50 mM phosphate buffer (pH 6.8). Enzyme assays were performed spectrophotometrically under a CO_2 gas phase by coupling the activity to NADH oxidation via exogenously-added malate dehydrogenase (for the PEP —> OAA direction) or pyruvate kinase/ lactate dehydrogenase (for the OAA —> PEP direction).

Results and Discussion

PEPCK was purified 42-fold with a 25% recovery of total activity. The isolated enzyme had an approximate pI of 4.1, a native molecular weight (as determined by MALDI- mass spectrometry) of 66.316 ± 0.07 kDa. SDS-PAGE revealed a single band having a subunit molecular weight of ~ 68 kDa, suggesting that the enzyme is a monomer.

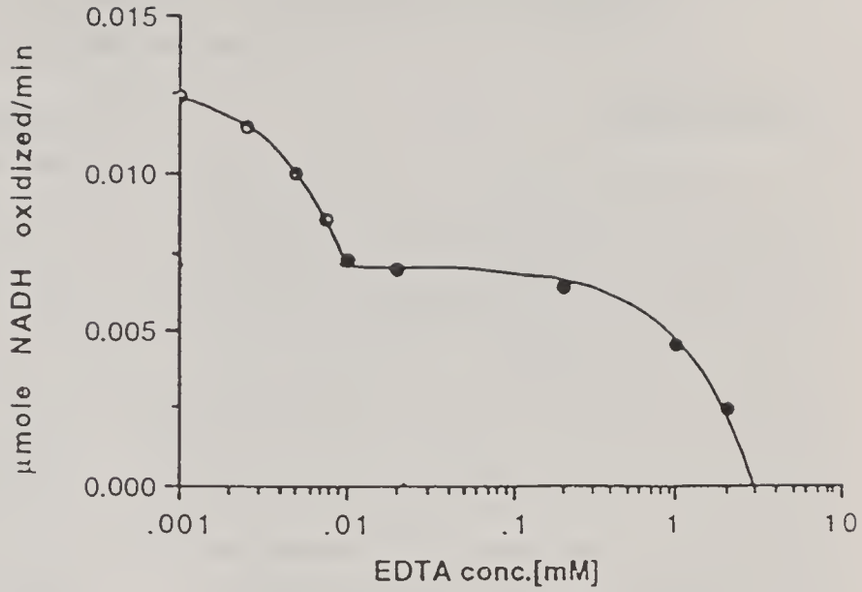


Figure 1. Biphasic titration of PEPCK activity by EDTA.

Activity in the presence of millimolar amounts of Mg^{++} was enhanced by addition of micromolar amounts of Mn^{++} (but not Co^{++}, Ni^{++}, or Zn^{++}). Titration of enzyme activity with ethylene diamine tetraacetic acid (EDTA) (Fig. 1) revealed a biphasic profile, with about half of the activity removed at an added EDTA concentration of 0.02 mM, followed by a much more gradual loss of activity at higher EDTA concentrations. By analogy with other PEP carboxykinases, it appears that Mn^{++} acts by activating the enzyme while Mg^{++} acts by forming a stable complex with the substrate GDP. At saturating concentrations of PEP, GDP, and CO_2, half-maximal activation of the enzyme was observed at 0.0087 mM Mn^{++}.

With fully activated enzyme, linear Lineweaver-Burk plots were obtained for variable PEP concentrations at four fixed GDP concentrations, and for variable GDP concentrations at four fixed PEP concentrations. Replots of the intercepts yielded approximate v_{max} values of 18.6 µmol/min/mg enzyme and K_m values of 0.21 mM for PEP and 2.03 mM for GDP. These K_m values are substantially higher than those reported for PEPCKs from other biological sources. The enzyme reacted less effectively with ADP as an alternate phosphoryl acceptor (apparent K_m 9.8 mM), while IDP did not serve as a phosphoryl acceptor. Assay of the enzyme in the direction of oxaloacetate decarboxylation revealed apparent K_m values of 2.7 mM for OAA and 28.7 mM for GTP; comparison of values for the reaction in the two directions suggests that the physiological function of the enzyme is in fact the carboxylation of PEP. In reactions conducted under an N_2 gas phase with added $NaHCO_3$, addition of carbonic anhydrase resulted in a decrease in PEPCK activity, suggesting that the HCO_3^- anion is the active species in the carboxylation reaction.

Conclusions

Purified PEPCK of *R. flavefaciens* FD-1 displayed a similar pattern of activation by Mn^{++} displayed by PEPCKs from nonruminal sources. The enzyme differed from previously described PEPCKs from other organisms in that it was not activated by other transition metals and had a narrower range of nucleotide substrates and a relatively poor affinity for PEP and, particularly, GDP. Kinetic data suggest that the enzyme operates in the direction of PEP carboxylation. Based on PEPCK activities measured in extracts of cells obtained from cellulose-fed chemostats, it appears that rather high steady-state concentrations of GDP may be required to allow the enzyme to operate at rates consistent with the observed rates of succinate formation.

Production of Hexanoic Acid from Cellulose and Ethanol by Cocultures of Ruminal Cellulolytic Bacteria and *Clostridium kluyveri*

P.J. Weimer, W.R. Kenealy and Y. Cao

Introduction

Hexanoic acid (caproic acid) is potentially useful as a liquid fuel precursor. However, there are no practical methods for its biological synthesis from biomass materials. The nonruminal anaerobic bacterium *Clostridium kluyveri* is unique in the microbial world for its ability to produce hexanoic (HA) and butyric (BA) acids as major fermentation endproducts, using a novel biochemical pathway that involves addition of two-carbon units from ethanol and acetic or succinic acids. Because ruminal cellulolytic bacteria such as *Fibrobacter succinogenes* S85 or *Ruminococcus flavefaciens* FD-1 produce acetic and succinic acids as major fermentation endproducts, coculture of these ruminal species with *C. kluyveri* offers a potential route for production of HA from cellulosic biomass commingled with dilute ethanol waste streams.

Materials and Methods

F. succinogenes S85, *R. flavefaciens* FD-1, and *C. kluyveri* ATCC 8527 were grown individually or in combination in serum vials under a CO_2 gas phase. A modified Dehority's medium was used for growing S85 or FD-1 on Sigmacell 50 microcrystalline cellulose, or for growing 8527 on 200 mM ethanol and 50 mM acetate or succinate. Cocultures were grown in the same medium with both cellulose and ethanol, but without added succinate or acetate. Time course experiments were conducted in 1 L fermentors in batch mode with or without automated pH control.

Cultures were clarified by centrifugation and the supernatants analyzed for ethanol and fermentation acids by HPLC. Residual cellulose was determined by a modified neutral detergent method.

Results and Discussion

C. kluyveri grew in coculture with either *F. succinogenes* S85 or *R. flavefaciens* FD-1 only when the cellulose-containing growth medium contained ethanol. Production of both HA and BA occurred following production of acetate and succinate intermediates and was greatly enhanced by controlling the pH of the fermentation (Fig.1). Cocultures of 8527 with S85 generally gave a greater yield of HA

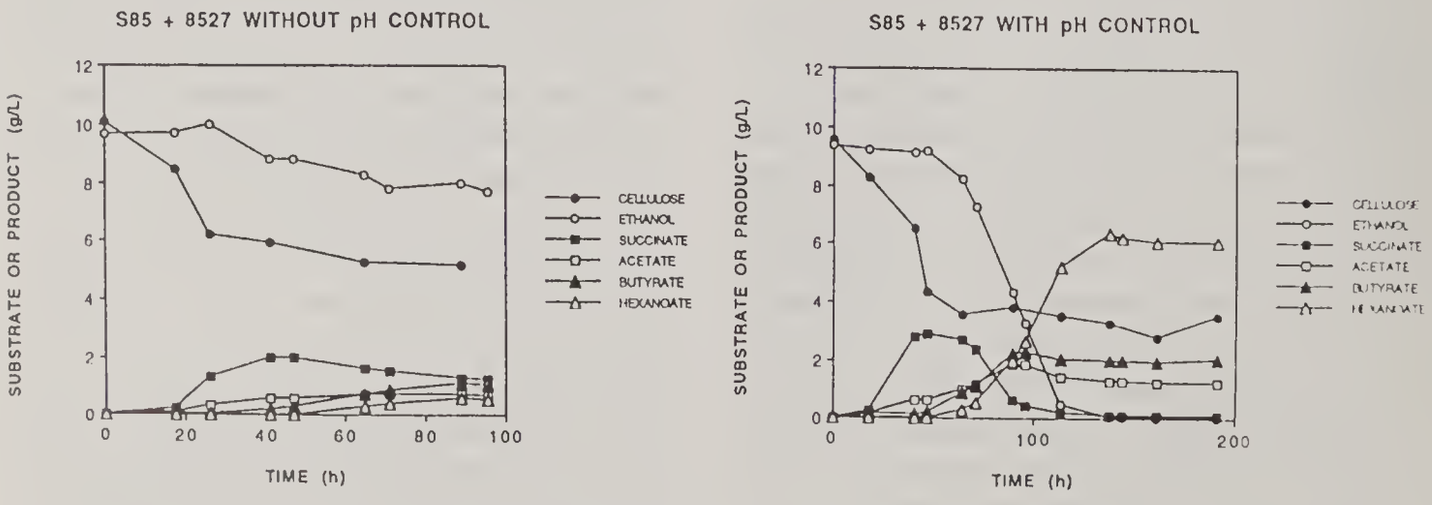


Figure 1. Time course of fermentation by cocultures of F. succinogenes S85 and C. kluyveri 8527. Top: no pH control; pH dropped from an initial value of 6.9 to a final value of 5.7 Bottom: pH controlled at 6.6.

than did cocultures of 8527 with FD-1, probably due to the higher succinate/acetate product ratio of S85. Experiments with pure cultures of the cellulolytic species revealed pH-dependent limitation in the rate of cellulose fermentation as hexanoate concentration in the medium increased, suggesting that the fermentation may be improved by continuous extraction of HA during the fermentation. By contrast, both the cellulolytic species and *C. kluyveri* tolerated relatively high concentrations (0.3 M) of ethanol without inhibition of growth or product formation.

Conclusions

Coculture of *C. kluyveri* with ruminal cellulolytic bacteria resulted in substantial conversions of cellulose and ethanol to mixtures of hexanoic and butyric acids. The process may be amenable to further development, particularly if the coculture can be expanded to include a third organism that generates ethanol directly by fermentation of the hemicellulose component of biomass.

Microbial Protein Synthesis in the Rumens of Cows Fed Alfalfa Silage, Alfalfa Hay or Corn Silage

A.N. Hristov and G.A. Broderick

Introduction

Microbial protein production plus feed protein escaping degradation in the rumen are the two sources of protein to the ruminant. Quantifying both is critical to proper diet formulation. An intriguing approach is to estimate microbial protein synthesis in the rumen of animals equipped only with ruminal cannulae. Walker and Nader (Aust. J. Agr. Res. 26:689, 1975) labeled microbial protein with ^{35}S and used the decline of radioactivity in bacterial S to estimate bacterial protein out-flow. Reliability of this method was limited by the fact that the two fractions of ruminal bacteria—those found in the fluid and particulate phases—were not separated. The stable isotope, ^{15}N, has been used to quantify microbial protein leaving the rumen, usually in animals fitted with cannulae in the rumen and abomasum or duodenum. The aim of our study was to measure microbial protein synthesis and protein degradation in the rumen on diets of alfalfa silage, alfalfa hay or corn silage in cows fitted with ruminal cannulae only.

Materials and Methods

Six multiparous, ruminally cannulated dairy cows averaging 619 kg BW and 198 DIM were blocked into two groups and fed three all-forage diets in a replicated 3X3 Latin square: alfalfa silage (AS) from first-cutting alfalfa ensiled at 38% DM, alfalfa hay (AH) harvested at 86% DM in small rectangular bales from the same fields as the AS, or corn silage (CS) ensiled at 36% DM. The CS diet was mixed with 2.2% urea and fed as a TMR. The AS, AH, and CS diets contained (DM basis), respectively, 20.9, 18.3, and 14.2% CP; 45, 48, and 39% NDF; and 39, 41, and 22% ADF. Diets were fed ad libitum for 4-wk periods (total 12 wk) with sampling during the last week of each period. To label microbial protein, ^{15}N was infused into the rumen as $(^{15}NH4)_2SO_4$; Cr-EDTA and Yb were infused as ruminal flow markers for liquid (L) and solid (S) phases. Non-ammonia N (NAN) in isolated bacteria, protozoa, L and S were analyzed for ^{15}N enrichment by isotope ratio mass spectrometry. Proportions of microbial

NAN in L and S NAN were computed from mean ^{15}N enrichments in L, S, and isolated bacteria. Proportions of bacterial and protozoal NAN derived from ammonia were computed from mean ^{15}N enrichments of ammonia and NAN in isolated bacteria and protozoa. Rumen emptying at 0- and 3 h after feeding was used to estimate NAN pool sizes.

Results and Discussion

As expected, highest ^{15}N enrichments and proportions of microbial NAN were found in L, with 63 (AS and AH) and 86% (CS) of total NAN estimated to be of microbial origin (Table 1). For S, this proportion was lower, ranging from 40 (AH) to 68% (CS). Greater proportions of microbial NAN may have been found in cows fed CS since feed NAN consumption was only 30 and 38% of that on AS and AH. Estimated proportions of bacterial NAN from ammonia ranged from 46 (AH) to 82% (CS) (Table 1). The proportions of protozoal NAN derived from ammonia for alfalfa—28 and 32%—were only 54 (AS) and 61% (AH) of those obtained on CS (Table 1). Lower NAN and greater NPN content probably gave rise to greater protozoal ^{15}N enrichment on CS-urea because protozoa were forced to depend for their own protein more on ^{15}N enriched bacterial NAN, and less on feed NAN.

Table 2 gives estimates obtained on the ruminal L and S NAN pools. Phase L represented only 17, 18, and 22% of the total NAN pool for the AS, AH, and CS. Although total NAN in L and S on CS was smaller, greater proportions of microbial NAN in both resulted in larger microbial NAN pools in L and S on CS (Table 2). Ruminal passage rate of L ranged from .150 (CS) to .169/h (AH) and did not differ with diet. Flow of microbial NAN with L also was not different among diets, averaging 120 g/day. Because of low NAN consumption, passage of feed NAN was lowest on the CS diet, about 35% of that on AS and AH. Ruminal passage rate of S ranged from .047 (AH) to .054/h (AS) and did not differ with diet. Although the amount of microbial NAN in S averaged three times that in L (Table 2), because of the much more rapid passage rate of L, microbial NAN flows in S and L were similar. On average, 70% of feed NAN escaping the rumen passed with S. Results from this study indicated that 39 (AS), 44 (AH), and 43% (CS without urea-N) of the NAN consumed in the diet escaped the rumen undegraded, estimates which were greater than published NRC (1989) degradabilities of 23 (AS), 28 (AH), and 31% (CS).

Feeding AH resulted in a total microbial NAN flow from the rumen (212 g/d) which was lower than on AS (235 g/d) and CS (243 g/d) (Table 2). The ^{15}N-enrichment of only bacterial NAN was used to compute microbial NAN flows from the rumen. The ^{15}N-enrichment of isolated protozoal NAN (Table 1) ranged from 54 (AS) to 75% (CS) of bacterial NAN. If protozoal NAN represented 25% (Leng and Nolan, J. Dairy Sci. 67:1072, 1984) of total microbial NAN outflow, then microbial NAN flow in our study would have been underestimated by 11 (AS), 10 (AH) and 6% (CS). Mean bacterial N predicted from NE_L intake (NRC, 1989) was 249 g/d, compared to our observed mean of 230 g/d of microbial NAN. Efficiency of microbial protein synthesis averaged 33 g NAN/kg OM digested in the rumen and was not different among diets (Table 2). Total NAN flows represented 74, 85, and 89% of total N intake for the AS, AH, and CS-urea diets.

Summary and Conclusion

Microbial NAN comprised 63 to 86% of total NAN in L and 40 to 68% of total NAN in S; the proportion was greater on CS than on AS and AH. An estimated 46 to 82% of bacterial NAN was derived from ammonia. Microbial NAN flow from the rumen was the highest for CS and lowest for AH, representing 50 (AS

and AH) and 76% (CS) of total NAN flow. Although microbial NAN in L averaged only 34% of microbial NAN in S, similar amounts of microbial NAN flowed with L and S. Mean ruminal pools of feed NAN were 14 g (L) and 105 g (S); however, 30% of feed NAN flowed from the rumen with L, suggesting soluble proteins escape to a disproportionate extent. An estimated 39 to 44% of NAN intake was degraded in the rumen.

Table 1. Steady state ^{15}N enrichment of ruminal NAN pools in liquid and solid phases, isolated bacteria and protozoa, and NH_3, and estimated sizes and origins of microbial NAN pools.[1]

Item	Alfalfa Silage	Alfalfa Hay	Corn Silage	SE	$P > F^2$
Bacterial NAN ^{15}N, atom % excess	.0454[c]	.0520[b]	.0569[a]	.0016	< .001
Liquid NAN ^{15}N, atom % excess	.0283[c]	.0328[b]	.0484[a]	.0010	< .001
Liquid microbial NAN,[3] % total N	63.1[b]	63.3[b]	86.3[a]	2.1	< .001
Solid NAN ^{15}N, atom % excess	.0283[c]	.0328[b]	.0484[a]	.0010	< .001
Solid microbial NAN,[3] % total N	40.9[b]	39.8[b]	67.9[a]	1.4	< .001
NH_3 ^{15}N, atom % excess	.0900[b]	.1460[a]	.0809[b]	.0133	.026
Protozoa ^{15}N, atom % excess	.0251[c]	.0317[b]	.0419[a]	.0013	< .001
Bacterial NAN from NH_3,[4] %	57.1[ab]	45.6[b]	82.2[a]	7.7	.038
Protozoal NAN from NH_3,[4] %	31.8[b]	27.5[b]	62.1[a]	5.8	.011
Protozoa : Bacteria ^{15}N ratio	.540[c]	.613[b]	.745[a]	.014	< .001

[a,b,c]Means having different superscripts differ ($P < .05$).

[1]Mean ^{15}N enrichments from 48 to 58 h after beginning $^{15}NH_3$ infusion.

[2]Probability of a diet effect.

[3]Microbial NAN in liquid or solid NAN, % = (^{15}N enrichment of liquid or solid NAN / ^{15}N enrichment of Bacterial NAN) x 100.

[4]Bacterial or Protozoal NAN from NH_3 N, % = (^{15}N-enrichment of Bacterial or Protozoal NAN / ^{15}N-enrichment of NH_3) x 100.

Table 2. Ruminal pools and flow rates of microbial and feed NAN in liquid and solid phases. Efficiency of microbial protein synthesis.[1]

Item	Alfalfa Silage	Alfalfa Hay	Corn Silage	SE	$P > F^2$
Liquid NAN, g	47.6[a]	47.3[a]	40.8[b]	3.3	.011
Liquid microbial NAN, % total N	63.1[b]	63.3[b]	86.3[a]	2.1	< .001
Liquid microbial NAN, g	30.2[b]	29.9[b]	35.1[a]	2.3	.006
Liquid feed NAN, g	17.4[a]	17.5[a]	5.7[b]	1.1	< .001
Liquid (Cr-EDTA) Slope, /h	.161	.169	.150	.015	.688
Liquid microbial NAN,[3] g/d	118.1	119.1	122.3	7.8	.732
Liquid feed NAN,[4] g/d	67.9[a]	69.5[a]	19.0[b]	3.4	< .001
Solid NAN, g	229.9[a]	212.4[a]	147.3[b]	9.9	< .001
Solid microbial NAN, % total N	40.9[b]	39.8[b]	67.9[a]	1.4	< .001
Solid microbial NAN, g	93.4[a]	84.2[b]	98.4[a]	4.2	< .001
Solid feed NAN, g	136.5[a]	128.2[a]	48.9[b]	6.4	< .001
Solid (Yb) Slope, /h	.054	.047	.050	.001	.082
Solid microbial NAN,[3] g/d	116.8[a]	92.9[b]	120.1[a]	5.4	< .001
Solid feed NAN,[4] g/d	169.4[a]	141.8[b]	59.3[c]	6.3	< .001
Total microbial NAN flow, g/d	235.0[a]	212.2[b]	242.5[a]	10.4	.002
Total feed NAN flow, g/d	237.3[a]	211.5[b]	78.3[c]	6.4	< .001
Total NAN flow, g/d	472.3[a]	423.7[b]	320.7[c]	16.3	< .001
Microbial NAN per DOM_r,[5] g/kg	31.9	33.0	32.8	3.3	.971

[a,b,c]Means having different superscripts differ ($P < .05$).

[1]Means from ruminal dumping measurements taken at 0- and 3-h after feeding.

[2]Probability of a diet effect.

[3]Liquid or solid microbial NAN flow (g/day) = Microbial-NAN in liquid or solids (g) x Cr-EDTA or Yb Slope (/h) x 24 (h/d).

[4]Liquid or solid feed NAN flow (g/day) = Feed-NAN in liquid or solids (g) x Cr-EDTA or Yb Slope (/h) x 24 (h/d).

[5]Efficiency of microbial NAN synthesis (g/kg) = Total microbial NAN (g/d) / organic matter digested in the rumen (kg/d).

Estimation of Protein Degradability in Roasted Soybeans by Near Infrared Reflectance Spectroscopy

G.F. Tremblay, G.A. Broderick and S.M. Abrams

Introduction

Economic value of supplemental protein fed to dairy cows is determined partly by the amount which escapes degradation in the rumen but remains available for absorption in the small intestine. Heat-treating by roasting is being used extensively to increase ruminal protein escape of whole soybeans. The non-enzymatic Maillard reaction, which occurs during heating, reduces microbial protein degradation without major change in intestinal protein availability. The dairy industry needs a rapid, inexpensive and accurate method to determine if commercially roasted soybeans (RSB) have been heat-treated to an optimal extent. An inhibitor in vitro (IIV) system developed by Broderick (Brit. J. Nutr. 58:463, 1987) provided a reliable procedure for estimating rate and extent of protein degradation in the rumen. The protein dispersibility index (PDI) has potential for identifying extent of heat-treatment for soybeans [Hsu and Satter, J. Dairy Sci. 74(suppl. 1):178, 1991]. However, both methods are too costly and time consuming for routine testing of RSB. Methods based on near infrared reflectance spectroscopy (NIRS) are now used widely to estimate chemical composition of forages and other commercial livestock feeds. The purpose of this study was to determine if NIRS can be used as a rapid method for predicting IIV protein degradability of RSB.

Materials and Methods

Roasted soybean samples (n = 266) were collected from various commercial sources in Wisconsin, Minnesota and Michigan. Wiley milled (1 mm) samples were analyzed for dry matter (DM) at 105°C for 48 h, total N by the Dumas method, PDI (Hsu and Satter, 1991), and rates of ruminal protein degradation using the IIV system (Broderick, 1987). Ruminal protein escapes were estimated using IIV degradation rates, assuming ruminal passage rate of .06 /h. Ruminal protein escapes corrected by regression (RPECR) were obtained using three reference proteins (casein, solvent soybean meal, expeller solvent soybean meal) in each run and were expressed on a DM basis. Samples were Udy cyclone milled (1 mm) and the NIRS spectra obtained utilizing an NIR System 6500 spectrophotometer (Perstorp Analytical, Silver Spring, MD). Scans from 121 samples (calibration set) were used to develop calibration equations for predicting DM, N, PDI and RPECR. Equations selected based on calibration statistics were used to predict DM, N, PDI and RPECR of the other 145 RSB samples (validation set) to evaluate the accuracy of calibration equations.

Results and Discussion

Mean (± SE) N content (DM basis) for the 266 RSB samples was 6.73% ± .02 and ranged from 5.29 to 9.04%. Mean (± SE) PDI for the 266 RSB samples was 13.5 ± .3. According to the classification system of Hsu and Satter (1991), 43% of the samples were underheated (PDI ≥ 14); 35% were marginally heated (PDI = 12 to 13); and 21% were heated to an optimal extent (PDI ≤ 12). One percent of the samples (4 samples) had PDI values of less than 8.0. The value of using solubility to estimate ruminal protein degradability stems from the concept that readily solubilized compounds are digested to a greater extent than their less soluble counterparts. The PDI method is based on protein solubility in distilled water. Protein solubility can be a simple and useful technique to assess treatment effects within protein sources, such as RSB,

but may lead to serious error when applied across diverse groups of feeds.

The inhibitor in vitro system (Broderick, 1987) quantifies extent of protein degradation from ammonia and amino acid accumulation in ruminal inoculum to which inhibitors have been added to allow quantitative recovery of these protein breakdown products. This method appears more sensitive to differences in fractional rate and extent of protein degradation for feedstuffs than solubility and ficin methods (Broderick, 1987) and in situ methods (Faldet et al., J. Dairy Sci. 74:2548, 1991). Therefore, the IIV procedure was used to estimate the fractional rate and extent of ruminal protein degradation for RSB. Mean (± SE) RPECR (CP basis) for the 266 RSB samples was 56.6% ± .4 and ranged from 33 to 77%. Only .8% (2 samples) were between 30 and 39% protein escape, 8% were between 40 and 50, 62% were between 50 and 60, 25% were between 60 and 70, and 5% were between 70 and 80% protein escape. Data for RPECR in Table 1 are expressed on a DM basis.

Values for PDI and RPECR (determined using the IIV system) were not well correlated (r^2 = .26). Protein solubility is confounded by the amount of N in the NPN and indigestible N fractions (Broderick, 1987). The coefficients of determination (r^2) obtained from NIRS calibration (n = 121) for DM, N, PDI and RPECR values were .97, .99, .71 and .90, respectively (Table 1). Statistics obtained from validation (n = 145) of the NIRS equations also are in Table 1. Correlation coefficients (r^2) between NIRS and laboratory analyses ranged from a low of .52 for PDI to a high of .86 for DM and N, with the r^2 for RPECR (DM basis) being intermediate (.70). Standard errors of the NIRS predictions were higher than the standard errors of calibration. There has been widespread interest in exploiting NIRS in predicting the nutritive value of forages and other livestock feeds. Although NIRS methods are empirical, there are now many convincing reports indicating that NIRS will accurately predict the results of bioassays, such as ruminal in vitro dry matter digestibility (IVDMD), in a wide range of forages. Furthermore, it has been shown that NIRS estimates are at least as accurate as Kjeldahl estimates for determining N concentration of soybeans.

Summary and Conclusion

These results indicate that NIRS can be used to predict DM, total N and, notably, ruminal protein escape of RSB.

Table 1. Calibration and validation statistics for near infrared spectroscopic (NIRS) analysis of roasted soybeans.

	Laboratory method		NIRS method		
Item[1]	Mean	SE lab	Mean	SE NIRS	r^2
Calibration (n = 121)					
DM, %	94.64	1.10	94.61	.26	.97
N, % (DM basis)[2]	6.70	- - -	6.71	.05	.99
PDI, % (total N basis)[2]	13.53	- - -	12.83	1.60	.71
RPECR, % (DM basis)	23.49	1.28	23.64	.98	.90
RPECR, % (CP basis)	56.10	- - -	56.37	- - -	- - -
Validation (n = 145)					
DM, %	94.51	1.03	94.64	.63	.86
N, % (DM basis)[2]	6.75	- - -	6.73	.12	.86
PDI, % (total N basis)[2]	13.41	- - -	12.57	3.52	.52
RPECR, % (DM basis)	23.93	1.11	24.04	1.54	.70
RPECR, % (CP basis)	56.72	- - -	57.15	- - -	- - -

[1]DM = Dry matter; PDI = Protein dispersibility index; RPECR = Ruminal protein escape corrected by regression; CP = Crude protein.

[2]Replicates were not used in the laboratory determinations of N and PDI.

Protein in Alfalfa Hay Is Used With Greater Efficiency for Milk Production Than Protein in Alfalfa Silage

G. A. Broderick

Introduction

Typical dairy rations formulated from alfalfa forages contain substantial amounts of degradable protein. Evidence from in vitro (Broderick et al., J. Dairy Sci. 75:2440, 1992) and in vivo [King et al., J. Dairy Sci. 72(Suppl. 1):554, 1989] studies indicated that alfalfa hay (AH) had greater amounts of undegraded intake protein (UIP) than alfalfa silage (AS). In a previous trial (1992 USDFRC Res. Sum., p. 115), it was found that milk protein yield was greater when cows were fed second-cutting AH plus high moisture corn (HMC) than when fed AS plus HMC. Therefore, a follow-up study was conducted in lactating cows fed diets with all of the forage from either AS or AH. The objective of this trial was to confirm the earlier observation of greater efficiency of utilization of protein in AH.

Materials and Methods

First-crop alfalfa was cut on 6/1/92. The AS was wilted to 41% DM, chopped and stored in an upright silo; AH was harvested at 85% DM as small rectangular bales from alternate windrows in the same fields as the silage. Mean composition of the AS and AH forages as fed were, respectively (DM basis), 19.9 and 16.5% CP; 40 and 41% NDF; and 32 and 32% ADF. The AS contained 54.4% NPN (total N basis). Twenty multiparous cows 40 days in milk were blocked into five groups of four cows each with nearly equal stage of lactation and assigned randomly to balanced 4 X 4 Latin squares. Four cows had permanent ruminal cannulae and were used for ruminal sampling on the last day of each period. Diets were fed for 3-wk periods (total 12 wk) before switching; production and intake data were analyzed from the 2nd and 3rd wk of each period. The four diets fed in the Latin squares as TMR were (DM basis): 1) AS, 66% AS plus 33% HMC; 2) AH, 66% AH plus 33% HMC; 3) AS + FM, 66% AS, 30% HMC plus 3% ruminant-grade fish meal (FM); and 4) AH + FM, 66% AH, 30% HMC plus 3% FM. Diets were supplemented with 1.3% minerals and vitamins; all four diets contained about 1.6 Mcal NE_L/kg DM. The CP contents of the diets were (DM basis): 1) 16.8%, 2) 14.6%, 3) 18.2%, and 4) 16.0%. Apparent digestibility of DM, OM, NDF, and ADF was estimated from fecal grab samples using indigestible ADF as an internal marker.

Results and Discussion

Intake of DM was lower and body weight was lost on the two AS diets compared to the two AH diets (Table 1). Milk production was lower on AS than on the other three diets. Fat and lactose contents of milk were not influenced by diet; yields of fat and lactose were lowest on AS, highest on AS + FM and AH + FM, and intermediate on AH. Yields of protein and SNF were lowest on AS, next lowest on AH, highest on AH + FM, and intermediate on AS + FM. Adding FM, an effective UIP source, to the AS diet significantly increased milk protein content and increased protein yield by 120 g/day. Adding FM to AH did not alter milk protein content, although protein yield was increased by 40 g/day (Table 1). That UIP supplementation of AS resulted in greater response than on AH indicated that the silage diet was a poorer source of absorbed protein, despite being 3.4% higher in CP. Lower CP content of AH reflected the greater leaf loss during harvest of hay versus silage. Ruminal ammonia and total amino acid concentrations were much higher on the two AS diets (Table 2), probably because of both lower protein degradability

and lower protein concentration with the AH diets. Apparent digestibility of DM, OM, NDF, and ADF all were greater with AS than with AH diets. These results indicated that AH was a better source of absorbed protein than AS, but AS was a better energy source than AH. Nelson and Satter (1988 USDFRC Res. Sum., p. 81) observed greater milk production with AS versus AH when feeding diets which were probably limiting in energy supply. Milk urea was higher when FM was added to AS but not when FM was added to AH. Blood plasma urea tended to reflect dietary CP levels; blood glucose was not altered by diet (Table 1).

Summary and Conclusion

Results from this trial indicated that both DMI and milk production were lower on AS than on AH when diets containing (DM basis) 66% alfalfa and 33% HMC were fed. Supplementation with ruminant-grade FM increased yield of milk and all milk components on AS, but only protein and SNF on AH: FM increased protein yield 120 g/day on AS and 40 g/day on AH. Findings from this trial and a previous study (1992 USDFRC Res. Sum., p. 115) indicated that absorbed protein supply will be greater on AH, despite CP losses during harvest, but that energy supply will be greater on AS. Feeding UIP supplements is more critical on AS-based than AH-based diets.

Table 1. Effect of forage source and ruminant-grade fish meal on DMI, BW gain, production of milk and milk components, and concentrations of milk urea and plasma urea and glucose.[1]

Item	AS	AH	AS + FM	AH + FM	$P > F^2$
DMI, kg/d	22.0^{c}	24.6^{a}	23.2^{b}	24.6^{a}	< .001
BW gain, kg/d	-.38^{b}	.51^{a}	-.04^{b}	.45^{a}	< .001
Milk, kg/d	34.6^{b}	36.5^{a}	37.5^{a}	37.5^{a}	< .001
Fat, %	3.53	3.42	3.47	3.45	.804
Fat, kg/d	1.21^{b}	1.24^{ab}	1.30^{a}	1.29^{a}	.033
Protein, %	2.90^{b}	3.01^{a}	2.99^{a}	3.06^{a}	.008
Protein, kg/d	1.00^{c}	1.10^{b}	1.12^{ab}	1.14^{a}	< .001
Lactose, %	4.86	4.75	4.76	4.80	.490
Lactose, kg/d	1.68^{b}	1.73^{ab}	1.79^{a}	1.80^{a}	.005
SNF, %	8.52	8.50	8.51	8.62	.758
SNF, kg/d	2.94^{c}	3.10^{b}	3.19^{ab}	3.23^{a}	< .001
Milk urea, m*M*	5.4^{b}	4.8^{c}	7.0^{a}	5.0^{c}	< .001
Plasma urea, m*M*	6.6^{b}	5.6^{c}	8.4^{a}	6.4^{b}	< .001
Plasma glucose, mg/dl	62	60	59	61	.086

^{a,b,c}Means within trial having different superscripts differ ($P < .05$).
[1]BW = Body weight; AS = alfalfa silage; AH = alfalfa hay; FM = fish meal.
[2]Probability of a significant dietary treatment effect.

Table 2. Effect of forage source and ruminant-grade fish meal on pH and concentration of ammonia and total amino acids in ruminal fluid.[1]

Item	AS	AH	AS + FM	AH + FM	$P > F^2$
pH	6.41^{a}	6.18^{b}	6.33^{ab}	6.20^{b}	.046
Ammonia, m*M*	13.9^{a}	5.5^{b}	15.1^{a}	6.6^{b}	< .001
Total amino acids, m*M*	3.7^{a}	.6^{b}	3.6^{a}	.9^{b}	.008

^{a,b}Means having different superscripts differ ($P < .05$).
[1]AS = Alfalfa silage; AH = alfalfa hay; FM = fish meal.
[2]Probability of a significant dietary treatment effect.

Ruminal Degradability of Protein in Leaves and Stems from Samples of Alfalfa Germplasm

G.A. Broderick, Y.-G. Goh, R.R. Smith and D.K. Barnes

Introduction

Protein in alfalfa forages is extensively degraded in the rumen. Although degraded protein may be reincorporated into microbial protein, forage proteins can be wasted due to excessive ammonia formation. Results from feeding studies have confirmed that alfalfa protein often is utilized inefficiently by lactating dairy cows. However, genetic differences were found in the ruminal degradability of protein in the alfalfa species *Medicago sativa* and *M. falcata* (Broderick and Buxton, Can. J. Plant Sci. 71:755, 1991). Although estimated ruminal protein escape in *M. falcata* was 24% greater than *M. sativa*, significant differences in degradation rate also were detected among the accessions of *M. sativa*, suggesting that genetic differences in ruminal degradability are present in common alfalfa. A ruminal in vitro system was used to screen samples of leaves and stems from 50 entries of *Medicago* germplasm for differences in microbial protein degradability to assess whether alfalfa selection should emphasize degradability of protein in leaves or stems.

Materials and Methods

In vitro incubations were conducted to determine ruminal protein degradabilities in 79 samples of leaves and 76 samples of stems from 50 alfalfa accessions. Twenty-five accessions had two plot-reps each of both leaves and stems. These samples were a subset from a core collection of 200 accessions representing the range in chemical composition in 1100 alfalfa entries harvested and separated in 1989 at St. Paul, MN. Samples were analyzed for dry matter (DM) and total N. Rates of ruminal protein degradation (k_d), and fractions degraded at 0-h (fraction A) and potentially degradable (fraction B) were determined using the inhibitor in vitro (IIV) system described earlier (Broderick, Brit. J. Nutr. 58:463, 1987); each set of incubations was repeated four times. Net extent of ruminal protein escape was computed using the equation:

Estimated Protein Escape = B x $[k_p/(k_d + k_p)]$

where k_p, the ruminal passage rate, was assumed equal to 0.06 /h. Six additional samples of leaves and stems were assayed for protein degradability using both the IIV system and in situ methodology (Karges et al., J. Anim. Sci. 70:1957, 1992).

Results and Discussion

Overall mean N content (DM basis), fraction A (0-h degraded N; total N basis), degradation rate, and estimated ruminal escape were, respectively, 4.04%, 13.0%, .095/h, and 36% for leaves, and 1.89%, 16.1% .185/h, and 22% for stems (Table 1). Results were essentially the same for leaves and stems from the 25 entries which were equally represented in each of two harvests from two field plots. Correlation coefficients (r^2) between leaves and stems for degradation rate and estimated escape were, respectively, .249 and .311. Estimated ruminal escape of protein in leaves and stems from different alfalfa species were, respectively, 37 and 20% (3 entries of *M. falcata*), 39 and 23% (2 entries of *M. varia*), and 34 and 20% (45 entries of *M. sativa*). Overall CV for degradation rate was 38% for leaves and 29% for stems, indicating greater variability among leaves. All differences between leaves and stems were highly significant ($P < .001$). The finding that degradability of protein in stems was greater than that in leaves was surprising. Therefore, six separate samples of leaves and corresponding stems for which there was

sufficient material were subjected to both in situ and IIV degradability determinations. In these samples, there were no significant differences in IIV degradation traits between leaves and stems, but the in situ rate observed for leaves was greater than that for stems (Table 2). However, because the degraded fraction A in stems was 75%, versus 52% in leaves (Table 2), there were no differences in the in situ estimates of ruminal protein escape for leaves (10%) and stems (7%). Approximately two-thirds of the protein in alfalfa herbage is in leaves; genetic selection for reduced degradability of leaf protein may be a possible means of improving utilization of alfalfa protein.

Summary and Conclusion

The IIV ruminal degradation rates for alfalfa leaf protein averaged about half the rates for alfalfa stem protein and estimated escapes averaged 36% for leaves and 22% for stems. Escapes for leaf protein were slightly greater in *M. varia* and *M. falcata* than *M. sativa.* Correlations between IIV rates and escapes for leaf and stem proteins were low. In situ protein degradation rates for leaves were greater than stems but in situ escapes were not different because of larger fraction A in stems. It may be more valuable to screen alfalfa germplasm for differences in ruminal degradability of leaf protein only. In situ methods may not be useful for screening alfalfa germplasm.

Table 1. Comparison of ruminal inhibitor in vitro degradabilities of protein in alfalfa leaves and stems.[1]

	Plant Part			
Item	Leaves	(SD)	Stems	(SD)
N content (%)	4.04	(.36)	1.89	(.18)
Degradation rate (/h)	.095	(.036)	.185	(.054)
Fraction A (% CP)	13.0	(4.1)	16.1	(5.1)
Fraction B (% CP)	87.0	(4.1)	83.9	(5.1)
Estimated escape (% CP)	35.7	(8.5)	21.7	(5.5)
(n)	(79)		(76)	

[1]Significant differences due to plant part and accession were detected for all traits ($P < .001$).

Table 2. Comparison of ruminal inhibitor in vitro (IIV) and in situ degradation data for six samples of alfalfa leaves and stems.

	IIV		In situ	
Item	Leaves	Stems	Leaves	Stems
N content (%)	4.20[a]	1.84[b]		
Degradation rate (/h)	.107[b]	.130[b]	.236[a]	.147[b]
Fraction A (% CP)	9.6[c]	11.7[c]	51.8[b]	75.3[a]
Fraction B (% CP)	90.4[a]	88.3[a]	48.2[b]	24.7[c]
Estimated escape (% CP)	35.1[a]	28.8[a]	9.9[b]	7.4[b]

[a,b,c]Means within each row having different superscripts differ ($P < .05$).

Feeding Alfalfa and Corn Silage Diets to Dairy Cows
1. Effect on Milk Yield and Composition

T.R. Dhiman and L.D. Satter

Introduction

The objective of this study was to determine the milk yield response to different proportions of alfalfa silage (AS) and corn silage (CS) in the lactating cow diet.

Materials and Methods

Forty-five mature cows and 29 first lactation cows were randomly assigned before calving to one of three treatments according to calving date. Cows were fed 50% forage and 50% grain diets (DM basis). The forage portion of the diet was either AS (Trt 1), 2/3 AS + 1/3 CS (Trt 2), or 1/3 AS and 2/3 CS (Trt 3). The experiment started at calving and lasted until cows completed 44 wks of lactation. The ingredient and chemical composition of diets (Diets 1-6) are given in Table 1. Diets 1, 2, and 3 were fed until cows were 12 wks in lactation. After 12 wks, cows were switched to diets 4, 5, and 6 unless milk yield was above 85 lbs/d for mature and 65 lbs/d for first lactation cows. Alfalfa and CS had 20 and 8% crude protein, respectively. Diets were balanced for vitamins and fed as TMR once daily. Daily feed intake and milk yield were recorded. Milk samples were analyzed once weekly for composition. Milk production totals (unadjusted for milk fat content) for mature cows for the 305 day lactation for treatments 1, 2 and 3 were 21,148; 22,422 and 22,100 lbs and for first lactation cows were 17,911; 18,546 and 18,008 lbs.

Results

Results are shown in Figure 1. Cows fed 2/3 AS and 1/3 CS diets had higher milk yield. Persistency of milk yield was improved in mature cows when CS was added to the diet compared with alfalfa alone. Diets containing 2/3 CS decreased milk fat content in mature cows during early lactation. First lactation cows fed all AS diet had lower milk fat content compared with diets containing CS. Milk protein content was not different among treatments in mature cows.

Conclusion

Milk yield and milk composition results suggest that 2/3 alfalfa + 1/3 corn silage is the optimum proportion of these two forages for lactating cows.

Table 1. Ingredient and chemical composition of diets, % DM basis.

	Diet					
Ingredient	1	2	3	4	5	6
Alfalfa silage	50.0	33.0	17.0	50.0	33.0	17.0
Corn silage	0	17.0	33.0	0	17.0	33.0
High moisture ear corn	33.2	32.2	30.2	40.6	38.6	34.5
Soybean meal	0	5.0	10.0	0	7.5	10.0
Roasted soybean	9.0	5.5	2.5	3.0	0	0
Meat and bone meal	4.0	4.0	4.0	4.0	2.0	3.0
Fat (hydrolyzed animal fat)	2.1	1.4	.8	.7	0	0
Bicarbonate	0	.25	.5	0	.25	.5
Premix of Ca, P, Mg, and S	1.0	1.0	1.3	1.0	1.0	1.3
Trace-mineralized salt	.7	.7	.7	.7	.7	.7
NE_L, Mcal/kg DM	1.69	1.69	1.69	1.62	1.63	1.66
Crude protein	18.6	17.5	16.6	17.0	16.1	15.5
Undegraded protein	6.7	6.7	6.8	5.9	5.8	6.2

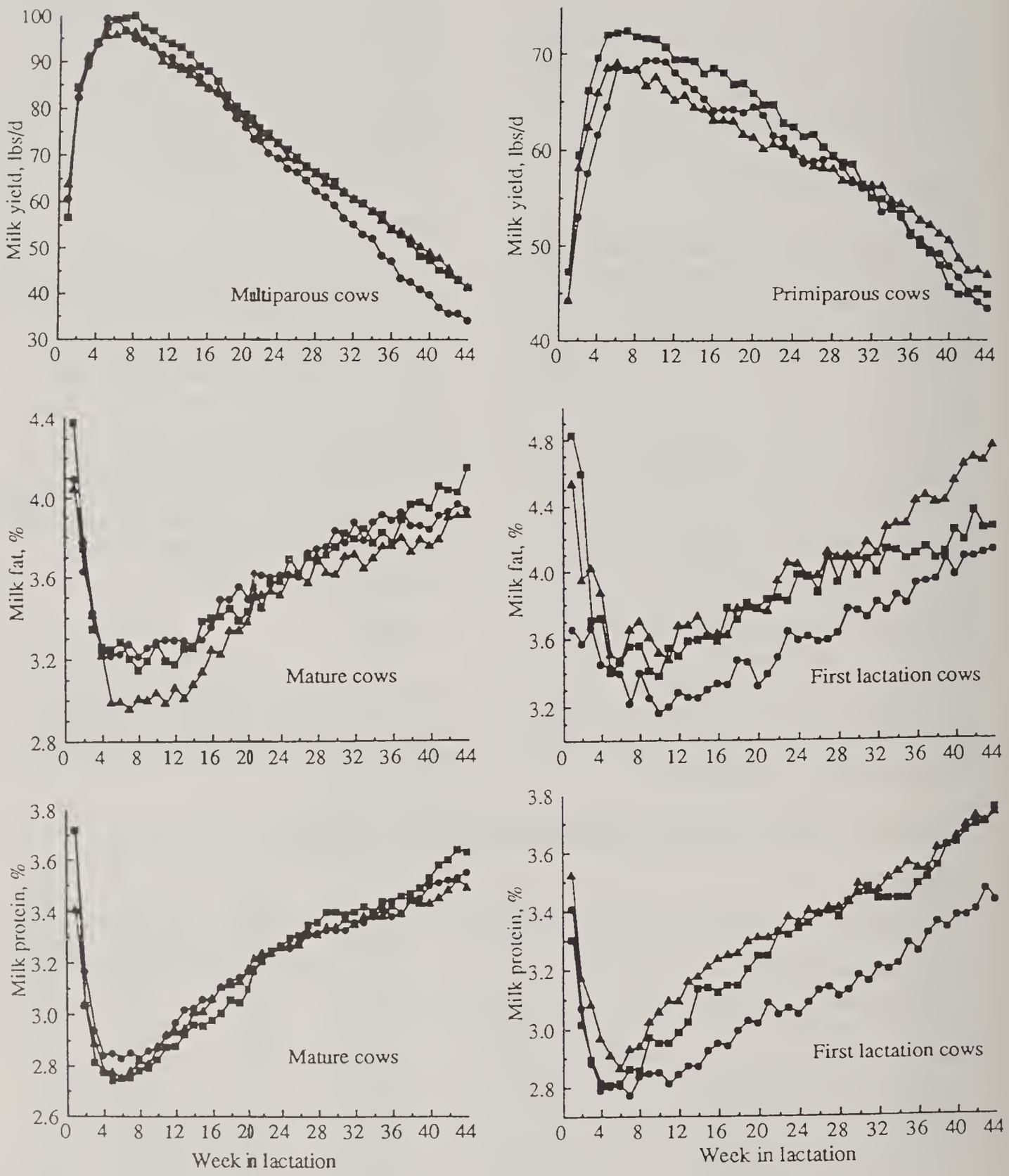


Figure 1. Effect of proportions of alfalfa silage and corn silage in the diet on rumen fermentation in diets with a forage to grain ratio of 50:50 on a dry basis. All alfalfa silage, -●-; 2/3 alfalfa + 1/3 corn silage, -■-; 1/3 alfalfa + 2/3 corn silage, -▲-.

Feeding Alfalfa and Corn Silage Diets to Dairy Cows
2. Effect on Rumen Fermentation Measurements

T.R. Dhiman and L.D. Satter

Introduction

Supply of rumen fermentable energy is important in cows fed good quality alfalfa silage (AS) because fermentable energy is required to match the rapidly degraded protein in alfalfa. Corn silage (CS), containing 40 to 45% grain is a good source of fermentable energy. The objective of this study was to determine the effect of diets containing a mixture of alfalfa and CS on rumen fermentation measurements.

Materials and Methods

Six rumen cannulated, mid lactation Holstein cows were used in a double 3x3 Latin square experiment. Each period was 21 days. The first 14 days were for adaptation, and measurements were made during the last 7 days in each period. Cows were fed 50% forage and 50% grain diets (DM basis). The forage portion of the diet was all AS (Trt 1), 2/3 AS + 1/3 CS (Trt 2), or 1/3 AS and 2/3 CS (Trt 3). Ingredient composition of diets (Diets 1-3) are given in Table 1 of the previous research report (*Page* 99). Alfalfa and CS had 20.3% and 8.2% crude protein (CP); 40.6 and 52.1% NDF; 34.4 and 25.9% ADF (DM basis), respectively. Diets were balanced for vitamins and minerals and fed as a TMR once daily. The CP contents of diets 1, 2, and 3 were 18.7, 17.6, and 16.7%, respectively. Feed intake and milk yield were recorded daily. Milk samples from four consecutive a. m. and p. m. milkings were analyzed for composition. Rumen fluid samples were

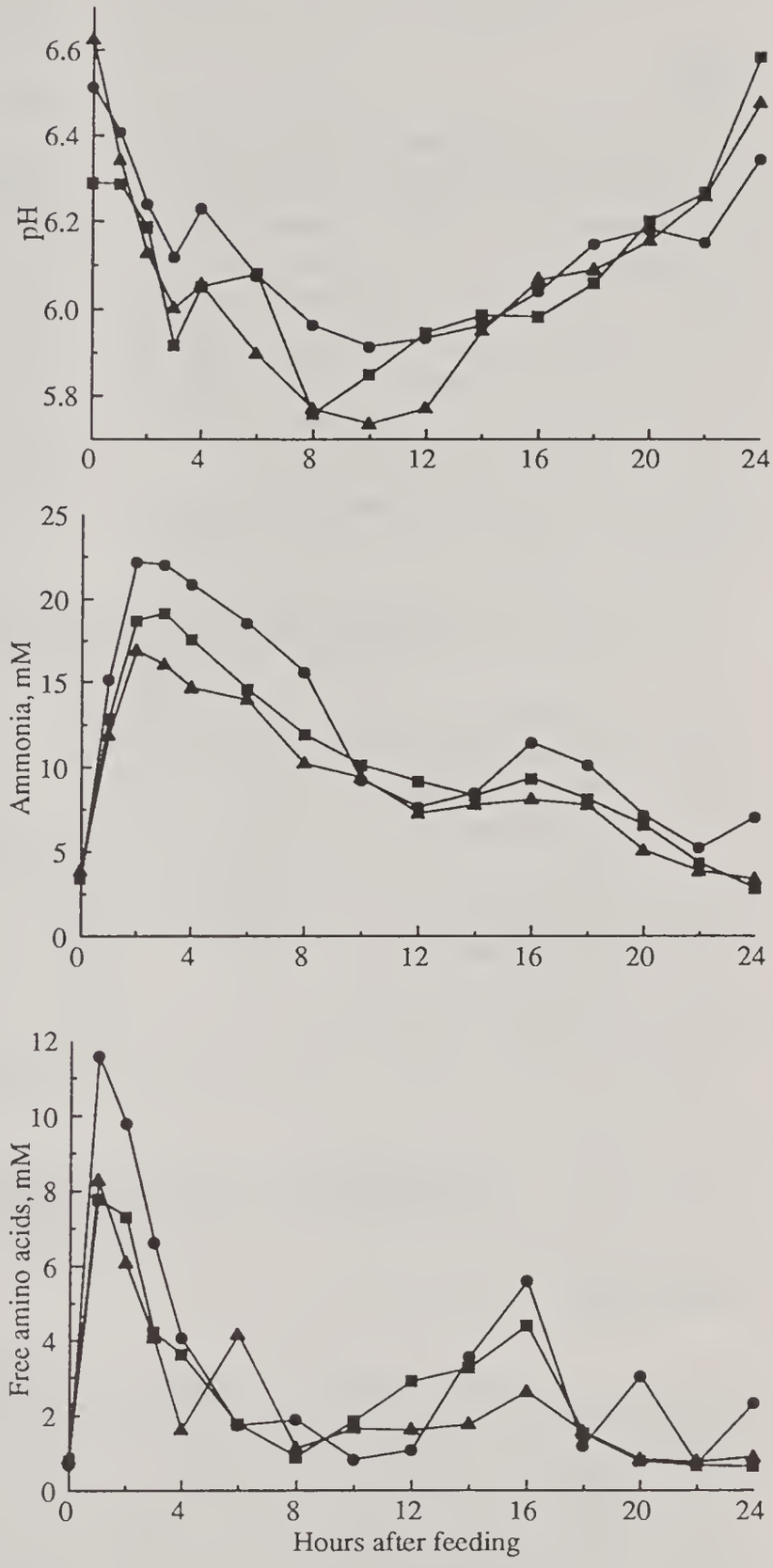


Figure 1. Effect of proportions of alfalfa silage and corn silage in the diet on rumen fermentation in diets with a forage to grain ratio of 50:50 on a dry basis. All alfalfa silage, -●-; 2/3 alfalfa + 1/3 corn silage, -■-; 1/3 alfalfa + 2/3 corn silage, -▲-.

collected during the last two days in each period. Rumen pH was recorded immediately after collection, and samples were preserved for further analysis of ammonia, amino acids, and volatile fatty acids.

Results

Feed intake, milk yield, and milk composition did not differ among treatments (Table 1). Daily patterns of ruminal pH, ammonia, and free amino acid concentration are shown in Figure 1. The average ruminal pH, ammonia, and free amino acid concentration in Trts 1, 2, and 3 were 6.15, 6.1, and 6.1; 12.3, 10.5, and 9.36 mM; 3.64, 2.83, and 2.52 mM, respectively. Diets containing CS had a lower ruminal ammonia and free amino acid concentration compared with alfalfa alone. Despite a lower dietary CP content of diets containing a mixture of AS and CS, ruminal ammonia concentrations were sufficient for microbial growth, and cows produced the same amount of milk with similar composition as with a diet containing AS alone.

Conclusion

The different proportions of corn silage and alfalfa silage in the three treatments had relatively little effect on the rumen fermentation. Ruminal concentrations of ammonia and free amino acids were lower as corn silage was incorporated into the diet, and this may have reflected the lower amount of dietary protein in the corn silage diets as well as better utilization of nitrogen in the rumen.

Table 1. Feed intake, milk yield, and milk composition.

	Treatment				
Parameter	1	2	3	SEM	*P*
Feed intake, kg/d	28.2	29.6	29.8	.7	.29
Milk yield, kg/d	39.7	39.1	39.8	.7	.74
3.5% FCM, kg/d	37.8	36.3	37.0	1.1	.65
Milk fat, %	3.28	3.08	3.18	.17	.73
Milk protein, %	2.97	3.06	3.09	.04	.17
Milk lactose, %	4.83	4.84	4.78	.03	.39
Solids not fat, %	8.57	8.66	8.63	.06	.63

Models for Describing Digestion Kinetics

D.R. Mertens

Introduction

Digestion is a multi-step process that can be understood more easily when it is divided into distinct mechanisms. Most current models describe digestion as a simple first-order process (where the disappearance of substrate per hour is proportional to the amount of substrate present at that time). These exponential models describe digestion kinetics in terms of rate of digestion and potentially digestible fraction. In some models, a discrete lag time is added to describe the lack of exponential digestion during early fermentation times. Kinetic parameters (lag time, rate, and potential digestibility) of digestion models are important because they, not only allow us to describe digestion, but also allow us to measure and characterize the intrinsic properties of forage cell walls and other components that limit their availability to ruminants.

Methods

Many times mathematical models are adopted by finding an equation that fits the data and then developing a biological rationale for parameters in the equation. In this study, the opposite approach was used. The biology of the digestive system was described graphically and in terms of fundamental differential equations. Then these equations were solved to obtain the integral equations that can be used to describe the digestive process. In most models, digestion and flow of residues out of in situ bags was assumed to be first-order. Using this approach, the absolute rate of digestion of substrate could be classified into one of four types of first-order models (Fig. 1). These four types of models served as the basis for deriving subsequent models of digestion. Seven different models have been described and derived, but only three will be discussed in this report.

Results and Discussion

Model 1 assumes that digestion is first-order with an indigestible fraction (Fig. 2). Differential equations for Model 1 are:

$$dD/dt = - kd * D$$
$$dI/dt = 0;$$

where t = time, I = indigestible residue, D = potentially digestible residue, and kd = fractional rate constant of digestion. The integrated equation for this model is:

$$R(t) = Di*\exp(-kd*t) + I;$$

where R(t) = total undigested residue remaining at any time = t and Di and I are the potentially digestible and indigestible residues at t = 0. The integral equation can be converted to a linear function by rearrangement and logarithmic transformation:

$$\ln [R(t) - I] = \ln [Di] - kd*t;$$

where ln is the natural logarithm of the terms within the brackets.

Implicit assumptions of Model 1 are: (1) the potentially digestible and indigestible pools act as distinct compartments with homogeneous kinetic properties, (2) the fractional rate of digestion is constant and is an intrinsic attribute of the digestion system and substrate, (3) digestion begins instantaneously at time zero and continues indefinitely, (4) enzyme or microbial concentrations are not limiting, and (5) the absolute rate, or flux, of the reaction is proportional only to the amount of potentially digestible pool present at any time. The key to using Model 1 to describe digestion is measuring and subtracting the indigestible fraction from the total substrate to obtain the potentially digestible fraction. This is usually done by measuring the asymptotic plateau of digestion after long fermentation times (> 72 hours).

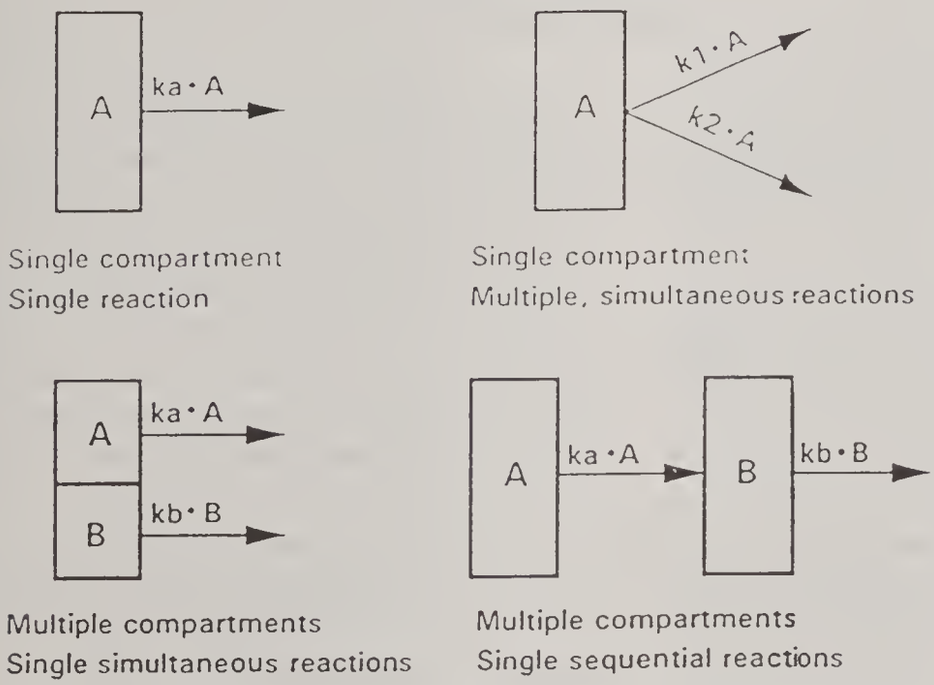
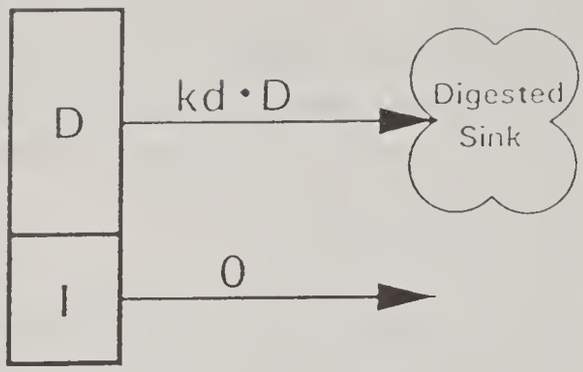


Figure 1. Illustrations of the various types of first-order models used to describe digestion.

Alternative models of digestion can be derived by building on the framework described by Model 1. Model 2 more adequately describes digestion of protein and dry matter by adding a soluble fraction to Model 1. Model 2 incorporated a discrete lag time



D = Potentially digestible fraction at any time
I = Indigestible fraction at any time
kd = fractional rate constant of digestion

Figure 2. Model 1 --- simple first-order digestion with an indigestible fraction.

into Model 1 to describe the delay in digestion that often occurs during early fermentation of fiber. Model 3 describes the lag phenomena as sequential first-order process (Fig. 3). The differential equations for Model 3 are:

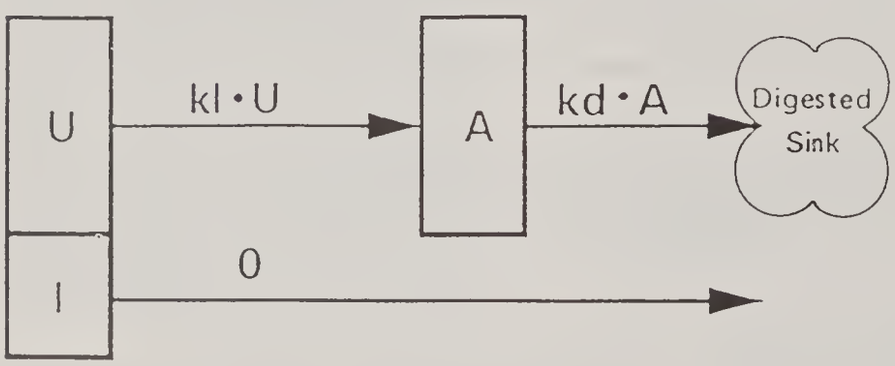
$dU/dt = - kl * U$

$dA/dt = kl * U - kd * A$

$dI/dt = 0;$

where, U and A are the potentially digestible pools that are unavailable or available for digestion, kl is the fractional rate constant for lag, and all other variables are defined as for Model 1. The integral equation for this model is: $R(t) = [U/(kd-kl)] * [kd*exp(-kl*t) - kl*exp(-kd*t)] + I.$

This model describes a smooth transition in digestion during the lag phase in contrast to the discontinuous function described by discrete lag models.



U = Unavailable potentially digestible fraction at any time
kl = Fractional rate constant of availability (lag phenomenal
A = Available potentially digestible fraction at any time
kd = Fractional rate constant of digestion
I = Indigestible fraction at any time

Figure 3. Model - sequential multi-compartmental model of digestion and lag with an indigestible fraction.

Model 5 was derived to describe digestion as a second-order process based on substrate and microbial concentrations. This model is analogous to the Henri-Michaelis-Menten kinetics developed for enzymatic reactions. Model 6 is a simple first-order model for in situ digestion that accounts for the influx and efflux of matter. Model 7 is a simple first-order model for describing the contamination of residues by microbial matter in in vitro systems (Fig. 4). These last two models demonstrate the complexity that can be incorporated into mathematical models to describe the biological process more accurately. The differential equations for model 7 are:

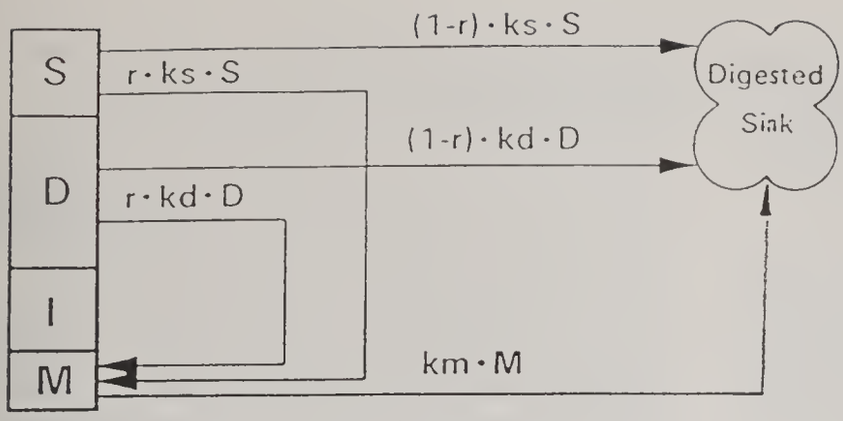
$dS/dt = -r*ks*S - (1 - r) *ks*S = - ks * S$

$dD/dt = -r*kd*D - (1 - r)*kd*D = - kd * D$

$dI/dt = 0$

$dM/dt = r*ks*S + r*kd*D - km*M;$

where, r is the proportion of digested matter converted to microbial matter, ks and kd are the fractional rate constants of digestion of soluble and potentially digestible insoluble matter, km is the fractional rate constant of microbial lysis, M is microbial matter in the in vitro vessel, and all other variables are defined as for Model 1. Assuming that a blank microbial matter is measured and subtracted, the integral equation for describing the dry matter remaining in the vessel is: $M(t) = D*exp(-kd*t) + I + \{[r*ks*(R0-D-I)/(km-ks)]*[exp(-ks*t) - exp(-km*t)]\} + \{[r*kd*D/(km-kd)]*[exp(-kd*t) - exp(-km*t)]\};$



S = Soluble fraction at any time
ks = Fractional rate constant of digestion for solubles
r = proportion of digested matter converted to microbial mass
D = Potentially digestible fraction at any time
kd = Fractional rate constant of digestion for insoluble matter
I = Indigestible fraction at any time
M = Microbial mass at any time
km = Fractional rate constant of microbial lysis

Figure 4. Model --- simple first-order model of digestion with soluble and indigestible fractions and contamination of residues by microbial debris that occurs when measuring the digestion kinetics of protein or dry matter using an in vitro system.

where, DM(t) is the dry matter in the vessel at any time = t, R0 is the total initial residue at time zero, and all other variables defined as above.

Conclusion

Rigorous mathematical derivation of models for describing digestive processes are beneficial for measuring intrinsic kinetic properties of feeds and for understanding the consequences of the biological principles involved. When used with adequate data and proper methods of fitting data to models, simple mathematical models can provide quantitative estimates of feed attributes that can lead to valuable insights into limitations in forage utilization. More complex models can overwhelm our ability to collect and fit data to these models, but still provide a rigorous basis for understanding biological processes and recognizing the limitations that are imposed on our understanding by simple models of digestion.

Developing a Cow Model Using Object-Oriented Programming - DAFOCOW Version 2.0

D.R. Mertens and R.E. Muck

Introduction

Use of computer models for describing and studying complex systems is often hampered by the lack of evolutionary development and refinement of models. In many instances, models are imbedded in computer programming code in a way that make it extremely difficult for anyone other than the originator of the model to decipher the code, improve it by adding modifications, or remove it to be used in other applications. Object-oriented programming (OOP) is a computer software design and programming approach that offers potential for enhancing the development and use of complex computer models or software.

The basis of OOP is the design and coding of objects. Objects have both properties and behaviors and in many ways mirror the real-world object they represent. This provides an advantage to the biologist who is developing the model because the computer code more closely simulates the real-world object. OOP removes a degree of abstraction from the design process and makes it easier to develop the computer program. For programmers and others wanting to use the model, OOP is advantageous because objects are private entities (analogous to software modules) that contain encapsulated data and methods that cannot be accessed by any other part of the larger program code without going through a carefully defined object structure. In addition to encapsulation (isolation of data and functions), OOP has the advantages of extensibility (overloading of operators so the same code can manipulate many types of objects),

inheritance (characteristics of one object can be transferred to another without rewriting code), and polymorphism (ability of different objects to respond differently to the same message).

The isolation of information and procedures in objects provides a highly modularized structure that facilitates the maintenance and reusability of computer code. This could lead to the development of an OOP library of computer objects that would be available to anyone wanting to mix and match specific components of the dairy-forage system to conduct systems research. The objective of this research was to develop an OOP cow model that would be usable by other scientists in a collaborative regional project to demonstrate the validity and value of the OOP approach to dairy forage systems research.

Methods

Borland's C++ was chosen as the programming language for developing the OOP cow model. Borland's applications libraries were used to provide the programming needed to handle data input/output and mathematical functions. The objects needed to describe the dairy forage system were defined by the members of the regional research project. Scientists at the US Dairy Forage Research Center agreed to develop the cow object for the project as a part of our plan to investigate the potential for OOP for use in future systems research at the Center. To facilitate the exchange of objects among scientists, a standard for coding objects is evolving within the regional project. The ZINC coding standard was adopted as the initial standard for programming style and is being modified as necessary to meet the specific needs of agricultural systems objects.

Results and Discussion

The current model (DAFOCOW v.2.0) is a transition between our first attempt at OOP (DAFOCOW v.1.0) and the version we plan to develop (DAFOCOW v.3.0) that will have digestive, metabolic, mammary, and reproductive subsystems as individual objects within the cow object. DAFOCOW v.1.0 was developed after attending a 3-day seminar on OOP for non-C++ programmers. This exercise suggested to us that C++ programming was within our capabilities and that the benefits of OOP would justify the effort needed to learn and apply this new approach to systems research.

DAFOCOW v.2.0 has the ability to simulate being offered feed, eating the feed, coming into estrus, being inseminated, becoming pregnant, calving, and producing milk, meat and manure. The object is designed to simulate the life cycle of a dairy cow from the time of first calving when given a milk production potential that is set by the modeler. The OOP cow model interacts with a GROUP_RATION object to simulate feed intake and production responses. DAFOCOW v.2.0 is a valid model of real cow output only when rations provide protein contents that meet or exceed requirements and when they contain energy (or fiber) levels that are within 15% of the animal's potential for production. Information available to the observer of the cow model (management object) includes feed intake, milk production and composition, body weight and nitrogen composition, pregnancy and lactation status, days in lactation, and manure amount and nitrogen composition.

Conclusion

The current OOP cow model (DAFOCOW v.2.0) contains the basic structure of a cow object that will evolve to meet the needs of the dairy-forage systems research effort at the US Dairy Forage Research Center. Experience in object-oriented design and programming has been gained that suggests that this approach is a viable way of developing models that will have the flexibility, reusability, and maintainability that is desired to achieve some of the goals of the systems research group.

A Pasture Simulation Model

V.R. Kanneganti and C.A. Rotz

Introduction

DAFOSYM (Dairy Forage System Model) is a whole-farm simulator in which major components of a dairy farm (weather, crops and soils, harvesting and machinery, feed storage, animal feeding, animal production and economics) are integrated to quantify component interactions and their effects on the system as a whole. The goal is to help identify management options and to evaluate alternative technologies on a dairy farm. Currently DAFOSYM does not have a pasture simulator. Evaluation of dairy farms with pasture as a component requires models of pasture growth that can interface with other dairy components. The objective of this study is to develop and validate a pasture simulation model.

Model Development

A schematic representation of the model is shown in Fig. 1. The model presented here is based on a grass growth model developed by Johnson and Thornley (1985). Crop states include leaf blade, stem (includes sheath), root, plant carbon (C) and N, and dead matter. Crop processes include photosynthesis, respiration, evapo-transpiration, root and shoot growth, senescence, and uptake of water and N. The crop maintains substrate C and N required for daily growth. Nitrogen is supplied through root uptake, and carbon through photosynthesis. Crop canopy is sub-divided into four compartments - growing leaves (GL), first (ML_1) and second (ML_2) fully expanded leaves, and senescing leaves (SL) representing leaf turnover in a grass tiller. Movement of leaf dry matter from GL to SL through ML_1 and ML_2 is modeled with a leaf turnover rate parameter adjusted for temperature and soil moisture effects. Movement of stem dry matter is modeled similarly. Soil processes are based on LEACHM, a model developed by Hutson and Wagenet (1991). Model soil states include soil water, organic N and C in humus and fresh organic matter, and inorganic N as NO_3, NH_4 or urea. Sorbed and solution phases for inorganic N are modeled using linear sorption isotherm. Soil processes include water and N flow, and N transformation. Richards' equation and convection-dispersion equation are used to describe water and chemical transport. Soil N transformations, consisting of mineralization, immobilization, nitrification, de-nitrification and volatilization among organic and inorganic soil N pools, are described by first-order rate parameters adjusted for substrate concentration, soil water content and soil temperature. Soil heat flux is modeled to estimate soil temperature.

Model Inputs

The model is developed as a common or "generic" model to simulate different pasture species. The specificity is obtained by incorporating species-specific parameters. As of now, these crop specific parameters include canopy light extinction coefficient, leaf and stem initiation rates, biomass partitioning coefficients for leaf and stem, and light saturated photosynthesis rate. Model requirements for user input include soil textural characteristics and daily weather data. The model simulates crop and soil processes daily and is coded in Fortran.

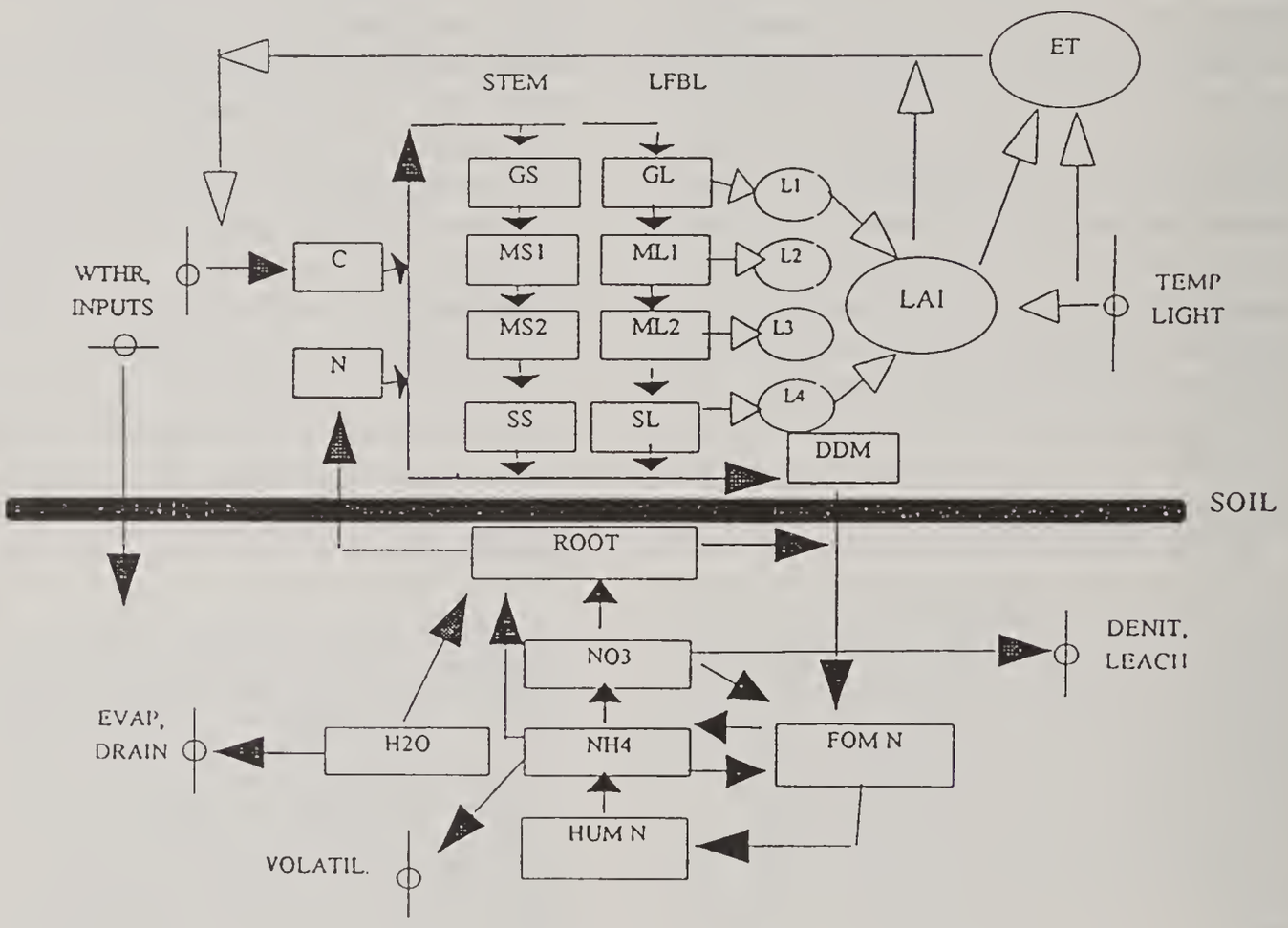
Model Validation

Parameters are being developed for different pasture species using data from the literature. After parameterization, the model will be validated and then incorporated into DAFOSYM.

Summary

A mechanistic, process-based perennial grass growth simulation model is developed to predict pasture growth as affected by plant genetics, weather and management. The model is developed with a vertical stratification of canopy to enable simulation of varying intensities of defoliation (grazing).

Figure 1. A simplified schematic representation of the pasture model. (Boxes represent model states; circles for auxiliary var.; filled and unfilled arrows for material and information flow, respectively.)



C = Substrate carbon, kg ha^{-1}; DDM = senesced dry matter, kg ha^{-1}; DENIT = N denitrified, kg ha^{-1} d^{-1}; DRAIN = Drained water, cm d^{-1}; PET = Evapotranspiration, cm d^{-1}; EVAP = Soil evaporation, cm d^{-1}; FOMN = Fresh organic matter N, kg ha^{-1}; GL, ML_1, ML_2, SL = Leaf blade compartments, kg ha^{-1}; GS, MS_1, MS_2, SS = Stem compartments, kg ha^{-1}; HUMN = Humus N, kg ha^{-1}; L_1, L_2,L_3, L_4 = Leaf area compartments, ha ha^{-1}; LAI = Leaf area index, ha ha^{-1}; LEACH = N Leached, kg ha^{-1} d^{-1}; N = Plant substrate nitrogen, kg ha^{-1}; NO_3 = Inorganic soil nitrate N, kg ha^{-1}; NH_4 = Inorganic soil ammonium N, kg ha^{-1}; ROOT = Root dry matter, kg ha^{-1}; VOLATIL = N volatilized, kg ha^{-1} d^{-1}.

Forage Available for Daily Intake from Natural Pastures Managed with Intensive Rotational Grazing

V.R. Kanneganti, R.P. Walgenbach and L. Massingill

Introduction

With increasing cost of production and declining farm income, dairy farmers are looking for ways of reducing input costs. Since grazing has the potential to lower feed costs significantly, farmers are seeking guidelines for incorporation of pastures into dairy farming. With abundant "natural" pasture land in northeastern and northcentral regions, farmers are interested in utilizing this resource with minimum inputs. The goal under these situations is to optimize pasture productivity with minimum inputs, rather than to maximize productivity with higher inputs.

While pasture growth was studied in detail under cutting (hay) management, similar data under grazing are limited, particularly for natural pastures in the region. Information collected under cutting management lacks plant-animal interface dynamics, and therefore, is not applicable for use under grazing. Pasture growth rate and forage intake under grazing are fundamental data required for estimating forage available for daily intake, so appropriate stocking rates and feed supplementation may be designed. By matching animal feed requirements with pasture production, wasteful overfeeding of supplements is minimized, thus reducing nutrient overloading on farms.

The objective of this study was to provide dairy producers practicing rotational grazing with quantitative estimates of forage available for daily animal intake across the grazing season.

Materials and Methods

Pasture used in this study is representative of "natural" pastures of northeast and northcentral U.S., with quackgrass and bluegrass as dominant species. No fertilizer or pesticide was used. Soils were adequate in available P and K. Pasture was divided into two blocks (replications), with 9 paddocks in each block. Each paddock measured about 0.3 acres and was managed with rotational grazing using dairy heifers. Average duration of a rotation/cycle was 28 days with 3 days for grazing on a paddock.

Pasture biomass was measured during each rotation, from May through September 1993, using double sampling technique. According to this technique, pasture biomass and canopy height relationship was developed for each rotation from 24 quadrats (3.3 ft. x 1.0 ft., each) harvested at random from 4 paddocks. Before harvesting a quadrat for biomass, canopy height was measured using a disc ("falling plate"). The disc was 1 ft. x 1 ft. square and weighed 0.5 lb. While the relationship between height and biomass was linear for all rotations, regression parameters varied significantly among rotations suggesting that biomass and height relationship varied across the season. In the second step of this technique, average pasture height for a paddock was determined from 45 height measurements taken at random across the paddock using the plate. Biomass was estimated from the corresponding regression equation as a function of average paddock height. Each paddock was sampled two times per rotation - before the animals were moved on to and after the animals were moved off the paddock.

Pasture growth rate was calculated as: $G_i = (W_b - W_a)/t_i$, where G_i is average pasture dry matter accumulation rate for rotation i (lb ac^{-1} d^{-1}), and $(W_b - W_a)$ represents pasture biomass accumulated over a regrowth duration of t_i days. Intake was estimated as the difference

between biomass measured before and after grazing.

Results and Discussion

Pasture growth rate as calculated in this study represents the amount of forage available for daily animal intake across the grazing season (Fig. 1). Pasture biomass accumulated at an average rate of 45 lb dry matter per acre per day during May through July and declined gradually to about 35 lb per acre per day by the end of August. Later growth rates declined sharply to about 14 lb per acre per day. It should be noted that July and August of this year were unusually wet, and therefore pastures may not have been stressed for soil moisture.

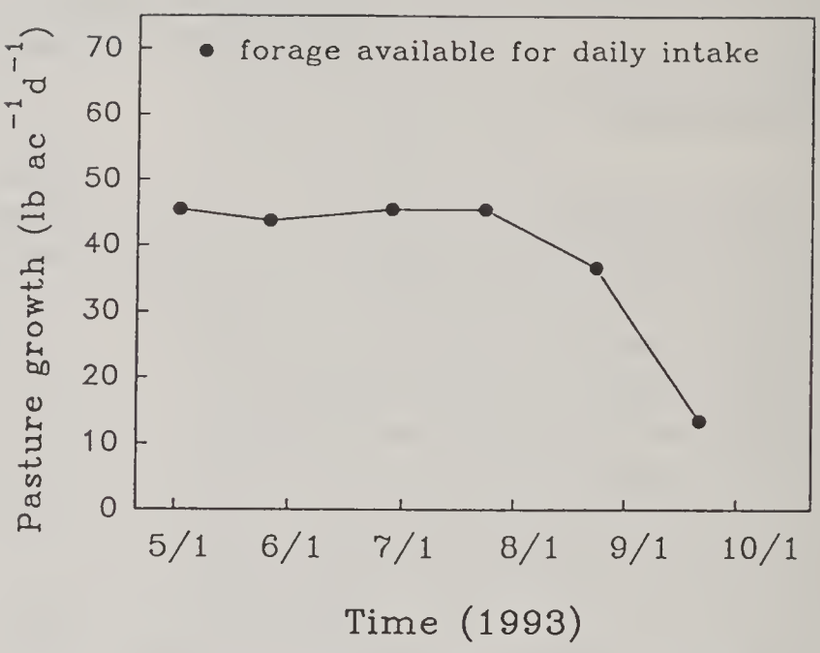


Figure 1. Pasture dry matter (d.m.) growth rate representing forage available for daily intake across the grazing season. (U.S. Dairy Forage Research Center Farm, Prairie du Sac, WI.)

Summary

Natural pastures managed with a 28-day rotation provided about 45 lb dry matter per acre for animal intake daily through August, and then the amount decreased to about 14 lb per acre per day by the end of September.

Using this information, a seasonal dairying program (240-270 day lactation, calving in spring) with different levels of feed supplementation under rotational grazing is being planned for next year. The main objective of this project would be to identify animal and agronomic managements (stocking rates, feed supplementation, animal health and breeding, pasture management, etc.) capable of supporting milk production levels (13000 - 18000 lb milk per cow per year) that can profitably sustain a family farm.

A Comparison of Grazing and Confined Feeding Systems on a Pennsylvania Dairy Farm

C.A. Rotz and J.R. Rodgers

Introduction

Production costs on dairy farms have increased more rapidly than the price of milk. This economic squeeze has caused farmers to look for ways to reduce feed costs. One option of interest is the use of rotational grazing. A thorough analysis and comparison of grazing and confined feeding systems must be made over many years of weather accounting for equipment, material and labor requirements, forage losses and feed supplementation. The dairy forage system model (DAFOSYM) provides a tool for such an analysis. DAFOSYM was used to compare the long-term performance and economics of grazing and confined feeding systems on a specific dairy farm.

Methods

DAFOSYM simulates the growth, harvest and storage of alfalfa, feeding of the herd and manure scraping, storage and spreading on a dairy farm. Model parameters were set to describe Plum Bottom Dairy Farm, a farm located near Belleville, PA. This farm successfully uses rotational grazing along with custom hired baling, chopping and manure hauling operations. The farm consists of 75 acres of tillable land plus 20 acres of permanent grass pasture. All tillable land is planted in alfalfa with a 6 to 8 year stand life. First, third and fourth cuttings are harvested as wilted silage and second cutting is baled in small bales. Sixty acres are harvested in first cutting with 44 acres harvested in later cuttings. The remainder is rotationally grazed with 87 acres grazed in the fall. Older stands used for grazing and the permanent pasture are overseeded with red clover. To model confined feeding systems, all alfalfa was harvested using the same four cutting strategy.

The herd consists of 40 Ayrshire and 20 Holstein milking animals plus 38 replacement stock. The average milk production is 18,555 lb/cow with 4% fat. In addition to pasture, animals are fed hay and a total mixed ration (TMR) consisting of alfalfa silage, ground shelled corn, soybean meal and minerals. The TMR is about 40% forage and 60% concentrate for the high lactation group. This was modeled in DAFOSYM using a minimum forage ration. When grazing, forage consumption is greater which was modeled using a maximum forage ration. With these assumptions, the model predicted feed use very similar to actual feed records for the farm.

The four systems compared included grazing and confined feeding systems with either custom hire of major operations or owned machinery for all operations. The same hay barn, silage bunker and manure storage were assumed for all systems. Grazing systems required additional investments in fence and watering equipment. Simulations were done for 25 years of weather. Prices were set to reflect the long-term relative values of farm inputs and outputs in 1993 dollars.

Results and Discussion

Use of rotational grazing along with good feeding management provided a substantial reduction in purchased corn, soybean meal and minerals on this farm. Their feeding strategy essentially uses pasture as direct replacement of TMR. The result is a 30% reduction in the annual corn and mineral consumption and a 20% reduction in soybean meal use through grazing (Table 1). Rotational grazing also improves the permanent pasture yield increasing total forage production.

The use of grazing provides a clear economic advantage for this farm (Table 1). Equipment and material costs increase due to the investment in fence and watering equipment. Fewer harvest and feeding operations, though, reduce fuel use about 25% and labor by 15%. The net of purchased and sold feeds decreases 16% through reduced use of corn and soybean meal. Grazing animals spend up to 70% less time in the barn, so about 30% less bedding is required with 30% less manure hauled each year (Table 1). Altogether, these effects provide a 15% reduction in the average feed and manure handling cost. Grazing is a little more economical with custom hired operations than with owned equipment (Table 1). The use of grazing does increase risk. The variations in feed and manure handling costs for the grazing systems over many years of weather are about double the variations of those costs with confined feeding.

Conclusion

With custom hired machinery operations, grazing reduced the total feed and manure handling cost about $1.00/cwt of milk. Since

milk production was similar among systems, this saving increased the net return over feed and manure costs by $182/cow or $114/acre of land. When all machinery was owned by the farmer, there was less benefit for not using those machines. Grazing still reduced costs by $0.86/cwt of milk, increasing net return by $161/cow or $101/acre.

Table 1. Average annual feed utilization, costs and net return for grazing and confined feeding systems with custom hired or owned machinery on a 60 cow dairy farm in Pennsylvania.

Production or economic result	Units	Grazing		Confined feeding	
		Custom	Owned	Custom	Owned
Preharvest alfalfa production	ton DM	253	253	361	361
Alfalfa hay production	ton DM	48	48	82	82
Alfalfa silage production	ton DM	154	154	212	212
Pasture consumed	ton DM	142	142	0	0
Corn grain purchased	ton DM	158	158	223	223
Alfalfa purchased (sold)	ton DM	19	19	(16)	(16)
Soybean meal purchased	ton DM	24	24	30	30
Minerals purchased	ton DM	5	5	7	7
Milk production level	lb/cow	18,555	18,555	18,555	18,555
Manure hauled	ton	1,600	1,600	2,300	2,300
Equipment and materials cost	$	8,664	19,375	7,460	18,990
Fuel and electric cost	$	1,107	1,692	1,493	2,327
Feed and manure storage cost	$	7,444	7,444	7,444	7,444
Labor cost	$	12,633	15,321	14,519	18,529
Custom operations cost	$	8,967	88	12,689	150
Seed, fertilizer and chemical cost	$	836	836	1,496	1,496
Net of purchased and sold feeds	$	28,028	28,028	33,458	33,458
Total feed and manure handling cost	$	67,679	72,784	78,559	82,394
Total feed and manure cost/unit milk	$/cwt	6.08	6.54	7.06	7.40
Milk income	$	145,057	145,057	145,057	145,057
Net return over feed and manure costs	$	77,378	72,273	66,498	62,663
Net return per cow	$/cow	1,290	1,205	1,108	1,044
Net return per acre	$/ac	814	761	700	660

Simulation to Evaluate Dairy Manure Systems

L.R. Borton, C.A. Rotz, H.L. Person, T.M. Harrigan and W.G. Bickett

Introduction

In recent years, larger herds and greater public awareness of environmental quality have caused manure handling on dairy farms to become a major concern. In making needed changes, manure management and its interactions with other parts of the dairy farm must be evaluated with a holistic, systematic approach. Computer modeling is required to integrate all major interactions on the farm from feed production to the return of nutrients back to the land for crop growth while tracking farm inputs and outputs for a comprehensive eco-

nomic evaluation. DAFOSYM provides a base for the development of such a model. The objectives of this work were: 1) to expand DAFOSYM to include the simulation of manure production, handling and application and 2) to illustrate the use of the model by comparing major options in manure storage and application on representative dairy farms.

Methods

An existing dairy forage system model (DAFOSYM) was expanded to model manure production, collection, storage, and application to crop land. The original model simulated the growth, harvest, storage and utilization of alfalfa and corn on a dairy farm over 26 years of weather. The revision allowed simulation of the quantity and nutrient content of manure produced as a function of the feeds fed, milk produced and animal growth. Manure dry matter (DM) was feed DM consumed minus the digestible DM extracted by the animals. Total manure included bedding, milking facility waste water and rain falling into the storage structure. Manure nutrients equaled the feed nutrient intake minus nutrients contained in milk and meat produced through animal growth. Nitrogen losses during collection, storage and application were each modeled to determine the amount of N applied to the soil. Of the N applied, a further reduction in availability due to the form of the N was modeled. Phosphorus and potassium losses were assumed to be 5% of that produced.

A wide variety of options in manure collection, storage, transport and application were modeled. Collection methods used hand scraping, gutter cleaners, a bucket scraper or loader or a collection pit and slurry pump. Bedding types were straw, sawdust and sand. Storage methods included a cement pad and buck wall for short term storage of semi-solid material, tanks for slurry storage and an earthen retention pond for liquid manure. Transport distance was variable with transport options of semi-solid and slurry tank spreaders, a slurry nurse tank and pumping of liquid manure. Manure was spread on the field surface, injected into the soil or surface applied through irrigation.

DAFOSYM follows a partial budget format which accounts for all costs associated with growing, harvesting, storing and feeding of crops to the milking herd and young stock and the collection, storage and application of manure back to the crop land. Costs of manure collection and application were determined from the predicted hours of machine use and labor, fuel and electricity used. These costs were reduced by a fertilizer credit determined as the value of the fertilizer replaced with manure nutrients. Manure was applied to each crop until the most limiting of the three major nutrients was met.

To illustrate the use of the model, six manure handling systems were compared on two synthesized, representative dairy farms. The smaller farm had 60 milking animals plus replacement heifers on 64 ha of land, and the larger farm had 250 milking animals plus replacements on 250 ha. Corn and alfalfa were grown on both farms with half of the land in each crop. The analysis was performed for 25 years of East Lansing, Michigan weather. Prices were set to reflect the long-term relative values of the various farm inputs and outputs in 1993 dollars.

Results and discussion

Slurry manure required the handling of nearly twice the mass of material compared to a semi-solid form. Machinery costs for the slurry system were 22% greater than for the semi-solid system; fuel costs were about 13% greater and labor requirements were 8% greater. Semi-solid material was not stored eliminating the storage cost. The slurry system retained more N allowing a small increase in the fertilizer credit. With all factors considered, a slurry system with six months storage

increased the net annual manure cost on the small farm by $71/cow. Injection of slurry manure into the soil better utilized manure N but increased handling costs compared to surface spreading. An increase in power requirement and a reduction in rate of application together caused a small increase in machinery and fuel costs. Overall, injection of manure increased manure handling costs and reduced the net return about $11/cow. Milk production level of the herd affected manure handling requirements and costs, but it had little effect on the comparison of systems.

Less difference among the three manure systems was found on the large dairy farm. All types of manure handling were more cost effective on the large farm compared to the small farm. The net manure cost for a slurry system with a six month storage dropped from over $202/cow on the small farm to $104/cow on the large farm. On the large farm, the slurry spread systems cost only $33/cow more than the semi-solid, daily haul system.

With surface spreading of manure, a delay of incorporation had only a small effect on system performance and costs. The major effect was a loss of nearly all volatile ammonia which reduced the N available for plant growth by 30%. On this farm, the loss of N increased fertilizer costs by about $900/year and decreased farm net return by $4/cow. These results indicate that the time between manure spreading and incorporation is not a major economic issue for this crop mix.

When manure transport distance was increased from 1 km to 5 km, machinery, fuel and labor costs increased dramatically. The total cost increase on the 250 cow farm was $52/cow which reduced the farm net return about the same amount. For a 5 km transport distance, the use of a nurse tank for transport and one spreader for application reduced manure handling costs by $42/cow. At a 1 km distance, total cost and net return were similar with and without a nurse tank. For a 1 km transport distance, manure irrigation reduced manure handling costs over $30/cow compared to transport and application with a slurry spreader. At a 5 km transport distance, the difference between slurry spreading and irrigation manure costs was $18/cow, but the cost was substantially greater than that of using a nurse tank for transport.

Counterflow Soybean Roaster

R.G. Koegel and T.J. Kraus

Introduction

Forage-based rations for dairy cattle normally require supplementation to bring protein and energy densities to a desirable level. The use of full-fat soybeans to increase both protein and energy levels has increased rapidly in recent years. Roasting of the soybeans is desirable, however, to (1) inactivate certain anti-quality substances (e.g. trypsin inhibitors) and (2) make the protein less subject to breakdown in the rumen.

The roasting process consists of three steps (1) heating, (2) "steeping" or holding at the elevated temperature, and (3) cooling to terminate reactions. Two types of roasters are used to roast most soybeans: (1) drum-type or concurrent flow in which the beans are introduced into the end of a rotating drum equipped with a burner; the drum axis is slightly inclined to the horizontal which causes the beans to slowly tumble toward the outlet end and exit as the drum rotates and (2) cross flow in which combustion gases from a burner are forced

through a shallow bed of beans as they are moved horizontally over a perforated deck by conveyor flighting. In both cases, additional chambers and conveying equipment must be added to carry out the steeping and cooling steps. This complicates the equipment and increases its size and cost.

The relatively short exposure time to the hot gases requires that the beans be exposed to high temperatures in order to transfer sufficient heat for adequate roasting. While the high surface temperatures of the beans have time to equalize with the low interior temperatures during steeping, there will always be a tendency to overtreat the exteriors in an attempt to adequately treat the interiors. Furthermore, the short exposure time between the hot gases and the beans leads to high exhaust gas temperatures, wasting energy to the environment. Another method of heating beans is with a screw expeller. In this process, mechanical energy is converted to heat by means of friction between the beans and the rotating screw. While the conversion from mechanical to heat energy in the screw expeller is efficient and heat addition to the beans uniform, the cost of mechanical energy is high relative to energy from fuels.

The objective of this research was to develop and evaluate an alternative roaster configuration with the following potential improvements:
(1) Reduction in energy requirement by means of lowering exhaust temperatures.
(2) Simplified machine configuration; reduced complexity and size.
(3) Maintenance or improvement of roasting uniformity: (a) between beans and (b) from exterior to interior of individual beans.

Material and Methods

The overall strategy for reducing energy requirements was to use a counterflow configuration, so that both the beans and the air leaving the roaster would be at the lowest practical temperature. This could be accomplished with a relatively simple roaster configuration by having the beans descend in a vertical column at a rate determined by an adjustable metering device at the bottom of the column. The column is divided vertically into approximate thirds with the upper third being the heating section, the middle third the steeping section, and the lower third the cooling section (Fig. 1). Air at ambient temperature is (1) forced upward through the cooling section, (2) allowed to bypass the steeping section via a passage where the air is heated and (3) then forced upward through the heating section. Since the exiting air passes through the cool, incoming beans, the potential for heat transfer is maximized.

A counterflow roaster with an approximate capacity of 1.8 t/h (2 tons/h; 67 bushel/h; 83 ft^3/h) was designed. While the original concept was to use two concentric vertical cylinders to form an annular column of beans, the roaster which was actually constructed was based on a modified grain dryer. The perforated vertical walls of this crossflow dryer were overlaid with non-perforated sheet metal and ducting added at the bottom to convert the crossflow dryer to a counterflow roaster. The external vertical walls were covered with insulation. Perforated panels were retained on the inner walls at the bottom and the top of the steeping section of the columns to allow most of the ascending air to bypass. It was expected that, since the flow resistance of the bypass was low relative to that in the steeping section, a large fraction of the air would follow this path. Gas burners in the bypass section or plenum add heat to the air prior to its reentry into the beans at the bottom of the roasting section.

A radial blade turbine blower (North American Manufacturing Company*, Designation 2416-F-15) with performance characteristics closely matching calculated requirements was chosen.

A reciprocating slotted metering plate at the bottom of the roaster controlled the rate of descent of the beans. Both the slot width and the dwell time at each end of the stroke were adjustable. Electrical linear actuators in conjunction with timers controlled motion of the plate. An auger below the metering plate removed beans from the roaster. A pressure sensor near the top of the roaster controlled an elevator and a horizontal auger to keep the roaster filled.

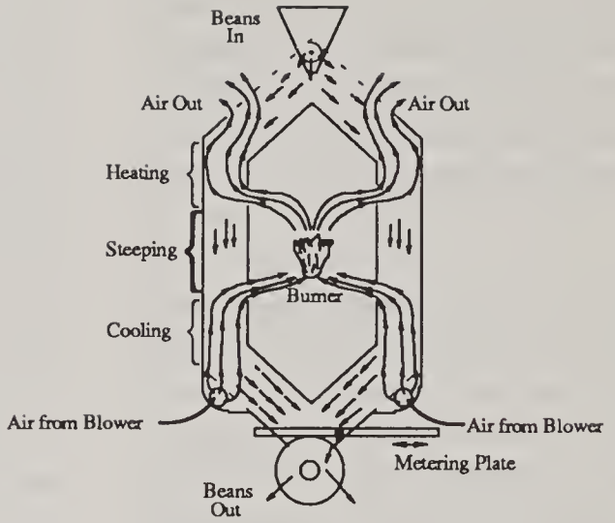


Figure 1. Schematic of counterflow soybean roaster.

The residence time in each of the three roaster sections was initially set to twenty minutes for a total time of passage of 60 minutes. A finite element heat transfer program, FEHT, was used to predict the time - temperature history at various points within the bean. This provided some basis for assessing whether the proposed temperatures and rate of passage were reasonable. Using properties from the literature and the assumption that the air to which any bean was exposed varied linearly from 38°C to 177°C (100°F to 350°F) over the 20 minute residence time in the heating section, the temperatures within the bean were calculated. According to this prediction, the surface and the center of the bean are only 2°C and 6°C, respectively, below the temperature of the air near the end of the 20 minute heating period. The temperature of the bean center lags that of the bean surface by less than one-half minute.

Results and Discussion

Evaluation and modification of the counterflow roaster are ongoing. Bean and air movement appear to be functioning as anticipated. The major problem encountered, so far, has been with the uniformity of treatment. In order to improve uniformity, changes have been made to the burner geometry and location, air flow and mixing within the plenum, and small cross ducts have been added between the inner and outer walls at the height where the heated air is introduced. These are made of perforated metal with an inverted "V" cross-section so that beans can easily flow over them. Work will continue to assure uniformity of temperature throughout the heating section.

Conclusion

The counterflow roaster appears to have the potential for saving energy and reducing roaster complexity. Quality and uniformity of the treatment are currently under evaluation and will determine the suitability of this design.

*Mention of a manufacturer's name is for descriptive purposes and does not constitute an endorsement.

U.S. Dairy Forage Research Center
Annual Dairy Operations Report, January 1994

L.L. Strozinski - Herd Manager

The past year has been a very challenging yet interesting and exciting time for us with a number of significant achievements and improvements. The Research Center herd count has increased by 50 to give a total of 620 animals (300 cows and 320 replacement heifers). We are currently milking 255 cows which are yielding an average of 67 pounds of milk per day. Our DHIA rolling herd average has increased from 18,390 pounds of milk, 642 pounds of fat and 569 pounds of protein to 19,235 pounds of milk, 706 pounds of fat and 597 pounds of protein. In 1993 the farm marketed 5.4 million pounds of milk.

Reproductive performance of the herd declined somewhat during the summer of 1993 but has rebounded this fall and winter. Average days open for the herd is currently 103 with a projected calving interval of 12.7 months. Our herd replacement heifers continue to enter the milking herd at an average weight of 1200 pounds at 24 months of age. Efforts are underway to reduce the average age at first calving to 22 months. First calf heifers now make up 35% of the milking herd. Average age of the milking herd has declined from 49 to 47 months.

The trend towards longer term research trials with larger numbers of animals has continued. A total of 330 animal units were used in ten trials in 1993. In addition, 140 cows were used in a cooperative reproduction research program with the University of Wisconsin Dairy Science Department. This project is studying the use of hormone injections to manipulate follicle development and synchronize estrous to facilitate artificial insemination by appointment.

Facility modification in 1993 has included expansion of the animal housing capabilities as well as the ability to obtain feed intake data on more animals. The increasing herd size has prompted the modification of one section of our large hay storage building to house bred heifers for the winter. Currently 28 animals are being housed in that unit. Thirty-six Calan feed gates have been purchased and installed in the mature cow free stall barn. These electronic feed gates will allow us to measure individual feed intakes of animals housed in a free stall system. This system will increase the number of animals on which we can obtain individual feed intake data from 142 to 178.

The 1993 summer season was the first season in which a pasture research program with growing dairy heifers was conducted at the center. Up to 100 heifers were grazed on ten plots established immediately north of the facility. Various stocking rates and rotational systems were used. Animal and pasture performance was evaluated. Plans are being made for pasture experiments with lactating cattle during the summer of 1994.

Overall, the Forage Center field facility has had a very productive year. There is no doubt that the majority of the operation's success must be credited to the hard work, dedication and cooperative efforts of both the field and dairy operation employees.

U.S Dairy Forage Research Center
Annual Field Operations Report, January 1994

R.P. Walgenbach

The 1993 growing season began similar to that of the 1992 season with great concern over winter survival of alfalfa. Early spring weather brought above normal rainfall and much colder than normal temperatures. These conditions contributed to loss of already winter damaged alfalfa in much of Wisconsin. Frequent and excessive rain characterized most of the growing season. In July, 9.18 inches of rain was recorded at the Research Farm and the period from May through August brought 25.2 inches of rain. Winter injury and the cold spring temperatures delayed the first alfalfa cutting and drastically reduced first cut yields. Corn and soybean planting proceeded slowly, and it seemed as though we were always planting when conditions were just too wet.

In spite of very difficult conditions, we managed to complete most plantings in a timely fashion. Several farms in the area had many fields that were left unplanted because of flooding. Corn planting was finished on May 29 and soybeans were finished on June 11. The last soybean field (60 acres) was planted after the first crop of badly winter damaged alfalfa was harvested. Several new alfalfa seedings (185 acres) were successfully established and appeared to be in excellent condition going into the winter season. Corn yields ranged from 107 to 185 bu/acre with most fields producing near 150 bu/acre. Soybean yields ranged from 33 to 55 bu/acre with most fields producing 40 to 45 bu/acre. Forage yields varied considerably from field to field. Highest yields were 5.3 tons/acre with most established fields producing 3.5 to 4.0 tons/acre. Sufficient forage was produced for the herd needs, but we will have very little carryover into the next season.

A shift was made this past season from growing winter wheat to growing high moisture barley which will be fed to the herd. I feel that this will improve our crop rotations and give us a little more flexibility in our crop planning. Excellent weed control occurred this past season, and only one alfalfa field needed spraying for leafhoppers. We continue to explore reduced and no-tillage practices and feel encouraged in moving towards these practices.

The chemical storage and handling facility was in full use this past season. We continue to purchase some of our leased equipment. A third John Deere tractor (85 h.p.) and a 12 foot New Holland mower conditioner were purchased this past season. We also purchased a Dyna drive unit to aid in our reduced tillage efforts. Bob Hager, our maintenance mechanic, was successful in acquiring three surplus 5 ton army trucks which have been mounted with tanks for manure hauling. This should help us become more efficient in handling our manure.

The installation of the electronic gate to replace the manned guard at gate 8 is nearly completed and should be fully operational by the beginning of our next cropping season. We have secured funding for renovation of our clay lined earthen storage lagoon. The design and planning have been completed, and work will be initiated as weather permits in March of 1994. The plan is to remove the existing clay liner which is about 1.0 foot thick and to replace this with 2.0 feet of clay, 5 inches of sand and 5 inches of concrete. The fuel contaminated soil removed from underground fuel tank sites is currently being remediated and will hopefully be fully decontaminated by this spring. The east side of our K3 hay storage shed was converted for use in winter housing of cattle to be used in grazing trials.

The weather this past season caused many disruptions and frustrations in our daily plans and operations. The field personnel responded to these situations in their usual professional and helpful manner. Under very stressful conditions they got the job done and done well. As I have stated in the past, these consistent efforts are a valuable contribution to our research programs and are much appreciated.

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